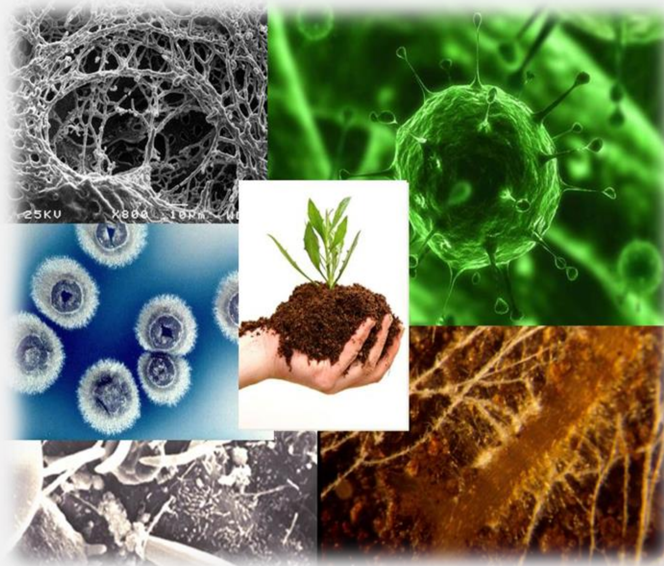
MICROBIAL β-GLUCOSIDASE GENES IN SOIL: MOLECULAR DIVERSITY, GENE EXPRESSION AND ENZYMATIC ACTIVITY



Shamina Imran Pathan

PhD Dissertation

Università degli Studi di Firenze

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Dedication

To Almighty God*; for making me able to achieve whatever I have.*

To My Husband, Imran Pathan*; this thesis is especially dedicated to you in very special way for your persistent support and care thorough out this journey.*

To my Parents*; who taught me to believe in myself, god and in my dreams. It is their unconditional love that motivate me to set higher targets.*

To my siblings; *for their support and motivation during my all life.*

To my family in Law; *for helping me when more I needed it.*

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Chapter 1: Introduction

1.1 The soil

Soil is acknowledged to be the “skin of the Earth” and it is end product of the weathering of rocks (parent material) and minerals under the effects of different biotic and abiotic factors including climate, macro and microorganisms, over time. Soil formation was quantified by the famous pedologist Hans Jenny (1941) which is called equation of Jenny;

$$S = f(Cl, O, R, P, T)$$

Where, S is Soil, Cl refers to reginal climate, O refers to potential Biota, R refers to relief, P refers to parent material and T refers to time.

The basic components of soil are minerals, organic matter, water and air. The average soil consists of approximately 45% mineral, 5% organic matter, 20-30% water, and 20-30% air, which is shown, in figure 1.1.

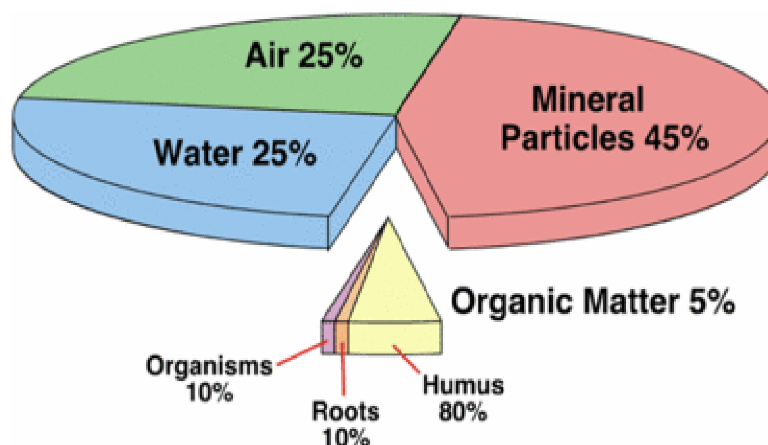


Figure 1.1 Typical soil composition

Soil is very complex and dynamic and its composition can fluctuate on a daily basis, depending on numerous factors such as water supply, cultivation practices, and/or soil type. Important soil physical and chemical properties are texture, density, organic matter content, pH values, soil stability, water holding capacity and overall soil fertility. Soil has three phases: the solid phase includes minerals and organic matters are generally stable in nature; the liquid and gas phases are water (a gaseous solution)

and air, respectively are the most influential properties of soils. The relative amount of air and water in soil are continually changing due to soil dry and wet cycles.

During soil formation process, soil particles are gradually reorganized as soil matures and forming distinct layers called soil horizons that are vary in thickness, mineralogical composition and organic matter content depending upon the paedogenesis process. The soil profile is a vertical section of the soil that depicts all of its horizons. The soil profile encompasses from the soil surface to the parent rock material. Soil horizons are signified with O, A, E, B and E horizons that are used for soil classification.

The O horizon is the surface horizon with organic material at various stages of decomposition and it is the most prominent in forested areas where there is the accumulation of plant debris. The A horizon is a surface soil layer mainly consists of minerals and substantial amounts of organic matter and generally predominant as surface horizon in grasslands and agricultural soils. The E horizon is a subsurface horizon, generally light in color and heavily leached. It is generally found beneath the O horizon and more common in forested areas. The B horizon is a subsurface horizon, accumulated from the upper layers and as deposition of certain minerals that have leached from the upper layers. The C horizon is a subsurface horizon. It is the least weathered horizon, known as the saprolite and it is composed of loose parent material.

Soil is made of single solid particles and generally, these particles stick together, forming aggregates, composed of organic and inorganic elements and define the microbial habitats. Aggregates are vary in different sizes ranges between µm to cm and these aggregates size mainly depends on land use and various environmental factors. Aggregate size distribution and shape control the formation of soil pores which are filled with either soil water or soil gases both affecting soil microbial activity, organic matter contents and redox conditions of soils.

1.2 Microbial diversity and soil functionality

The soil is highly heterogeneous and complex microhabitat with huge diversity of microorganisms and their versatile metabolomics activities (Sharma et al., 2014). Bacteria and fungi are main components of soil microflora and carry out almost all known biological reactions in soil. Thus, this microbiota plays an important role in soil fertility and involved in the main nutrient cycles (Nannipieri et al., 2003). Hence, soil microbiological properties are considered as more sensitive than chemical and physical properties to changes in management and environmental conditions (Lynch et al., 2004). Contribution of soil microbial diversity in various ecosystem services was shown in Table 1.1.

Microbial group	Process	Ecosystem service	Ecosystem service category
Heterotrophic bacteria/archaea	Organic matter breakdown, Mineralization	Decomposition, nutrient recycling, climate regulation, water purification	Support and regulation
Photoautotrophic bacteria	Photosynthesis	Primary production, carbon sequestration	Support and regulation
Chemo(litho)autotrophic	Specific transformations (e.g., NH_4^+, S_2^-, Fe_2^+, CH_4 oxidation)	Nutrient recycling, climate regulation, water purification	Support and regulation
Archaea	Specific elemental transformation (e.g., metals, CH_4 formation, NH_4^+ oxidation), often in extreme habitats.	Nutrient recycling, climate regulation, carbon sequestration	Support and regulation
Fungi	Organic matter breakdown and mineralization	Decomposition, nutrient recycling, soil formation, primary production (i.e., mycorrhizal fungi)	Support
Viruses	Lysis of hosts	Nutrient recycling	Support
Unicellular phytoplankton	Photosynthesis	Primary production, carbon sequestration	Support and regulation
Protozoa	Mineralization of other microbes	Decomposition, nutrient recycling, soil formation	Support
All	Production of metabolites (e.g., antibiotics, polymers), degradation of xenobiotics, genetic transformation, xenobiotics, genetic transformation, and rearrangement	Production of precursors to industrial and pharmaceutical products	Support

Table 1.1 Contribution of soil microbial diversity in various ecosystem services (Adopted from Bodelier et al. 2011).

Biodiversity is related to soil functions but mechanisms and influence of microflora on soil functions are still unclear (Andrén and Balandreau 1999, Turbé *et al.* 2010). Microorganisms are found in large

numbers in soil - usually between one and ten million microorganisms are present per gram of soil - with bacteria and fungi being the most prevalent but only up to 10% of microbes are cultivated; due to this reason, it is difficult to study their physiological characteristics. However, the availability of nutrients is often limiting microbial growth in soil and most soil microorganisms may not be physiologically active in soil at the given time. Therefore, we can assume that only some selected and adopted taxa are active in each environment (Turbé *et al.* 2010). It is essential to measure high number of enzyme activities and combining them into one single index to have information about soil microbial activities (Nannipieri et al., 2003).

The present enzyme assays measure potential activities rather than real enzyme activities because these assays are carried out at optimal pH and temperature, at saturating substrate concentration and soil as slurries and these conditions do not occur *in situ* (Nannipieri et al., 1990). Studying relationship between soil biodiversity and function can give indication on resilience or resistance of soil. As reported above, most of soil microbiota are still unknown because of difficulties in measurement of microbial diversity (Sharma et al., 2014). In addition, we usually measure soil functions by determining the rate of microbial process, without knowing which microbial species is effectively involved in the measure process (Sharma et al., 2014). Information on the entire soil microbial gene pool is of paramount importance to identify species living in soil and probably their involvement in the soil processes. The use of molecular techniques, which are based on direct extraction, and analysis of nucleic acid in soil can allow to determine the huge microbial diversity in soil. Involvement of high throughput sequencing methods integrated with the Stable Isotope Probes (SIP) approach can allow to analyse soil structural and functional diversity. However, it is important to determine proportion of expressed genes, which can be analysed by DNR/RNA ratio (Baldrian et al., 2012). Nevertheless, microbial diversity is very important for soil functionality; indeed microbial diversity also maintain resilience and stability, which is necessary to ensure soil functionality in different conditions. (Nannipieri et al., 2003; Turbé et al. 2010).

1.3 Soil enzymes

Enzymes catalyse almost all reactions in soil including organic matter decomposition and thus they play a vital role in maintaining soil health. Soils with different amount of organic matter also have different microbial activity and enzyme activities. Soil enzymes are mainly of microbial origin but plant and soil fauna also contribute at little extent. They are mainly categorized in two main groups: extracellular and intracellular enzymes. Enzyme which are occurring and functioning inside living cells are referred as intracellular enzymes whereas extracellular enzymes refer to enzymes capable of

coming into contact with substrate that are not incorporated into cells means, they catalyse reaction outside organisms (Ruggiero et al., 1996). The distribution of enzyme activity in soil is shown in figure 1.2.

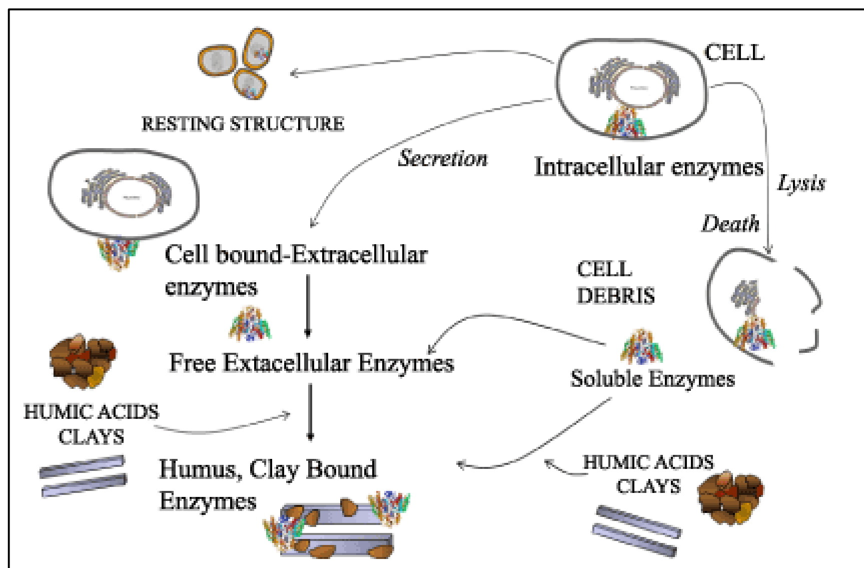


Figure1.2 Distribution of enzyme activity in soil (adopted from Rao et al., 2014)

Intracellular enzymes generally associated with cytoplasmic functions, play a key role in the microbial processes whereas extracellular enzymes are generally responsible for breakdown of polymers into monomers to be taken up by microbial cells. Extracellular enzymes can be free or immobilized on surface of soil particles (Gianfreda and Bollag, 1996) and named as stabilized enzymes (Gianfreda and Rao, 2011; Nannipieri et al 2012). Released extracellular enzymes are likely linked or attached to solid support such as clay, other minerals and organic matters and can be present as enzymes. In soil different enzymes are catalysing the same reaction can be produced by single organism. For example, Cañizares et al (2011) detected the three different sized proteins with β-glucosidase activity produced by *Pseudomonas putida*.

Soil enzyme activities can be affected by soil chemical and physical properties, organic, clay and microbial biomass, agricultural management, environmental pollutions, fertilizers, pesticides, salt heavy metal etc (Nannipieri et al., 2012). These factors directly or indirectly and reversibly or irreversibly can influence production of enzymes, their catalytic behaviours and their persistence in soil. Number of research and publications are available in literature on soil enzymes. However, there is always a consistent number of open and unsolved questions concerning extracellular enzymes such as the localization of stabilized extracellular enzymes in the soil matrix, their contribution to

substrates turnover and to biogeochemical processes, and their relationship with soil organisms (Rao et al., 2014; Nannipieri et al., 2012; Wallenstein and Weintraub, 2008).

Soil enzyme activities are of paramount importance to assess microbial functions, cycling of nutrients and carbon-sources decomposition. Thus, these catalysts provide a meaningful assessment of reaction rates for important soil processes. Therefore, soil enzyme activities can be used as measures of microbial activity, soil productivity, and inhibition of pollutants (Tate 1995). Combination of developing technologies with information gained by current techniques may permit the development of new and more microbially biogeochemical models that may be better predict the impacts of enzyme mediated soil processes (Rao et al., 2014). Therefore, enzymatic studies across different environments may greatly assist researchers to achieve a thorough comprehension "of the ultimate controls and biogeochemical consequences of extracellular enzymes across environments" (Arnosti et al., 2014). Involvement of different soil enzymes in carbon and other nutrient cycle are showed in table 1.2.

Enzyme	EC Number	Enzyme Reaction	Indicator of Microbial Activity	Process
Endoglucanase	3.2.1.4	Cellulose hydrolysis	C-cycling	Cellulose Degradation
Exoglucanase	3.2.1.91	Cellulose hydrolysis	C-cycling	Cellulose Degradation
β-glucosidase	3.2.1.21	Cellobiose hydrolysis	C-cycling	Cellulose Degradation
Endoxylanase	3.2.1.8	Hemicellulose hydrolysis	C-cycling	Hemicellulose decomposition
Arabinofuranosidases	3.2.1.55	Hemicellulose hydrolysis	C-cycling	Hemicellulose decomposition
β-xylosidase	3.2.1.37	Xylobiose hydrolysis	C-cycling	Hemicellulose decomposition
Endochitinase	3.2.1.14	Chitin hydrolysis	C-cycling	Chitin Degradation

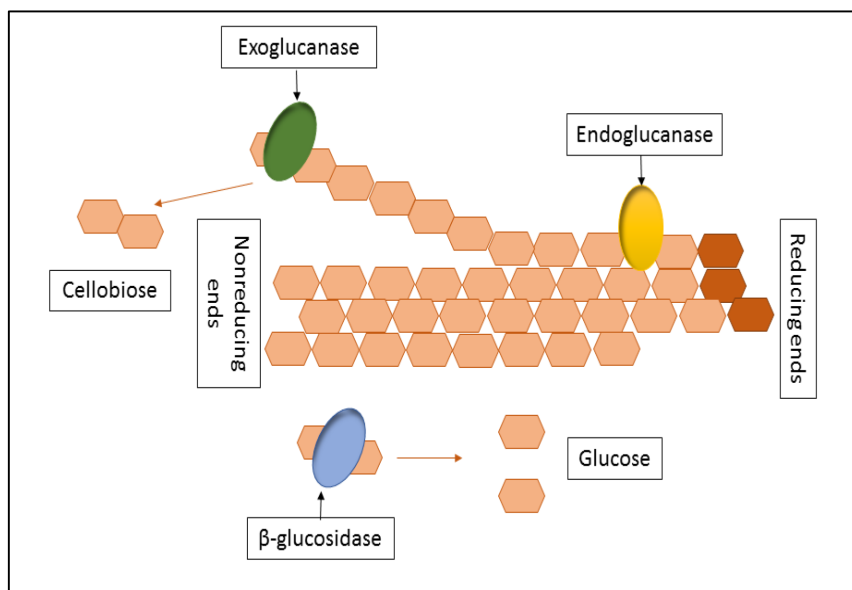
β-N-acetylglucosaminidases	3.2.1.30	Chitobiose hydrolysis	C-cycling	Chitin Degradation
Lignin peroxidase	1.11.1.14	aromatic ring oxidized to cationradical	C-cycling	Lignin transformation
Mn-peroxidase	1.11.1.13	Mn(II) oxidized to Mn(III)	C-cycling	Lignin transformation
Laccase	1.10.3.2	phenols are oxidized to phenoxyl radicals	C-cycling	Lignin transformation
Proteases	3.4.21.XX	Proteolysis	N-cycling	N acquisition
Urease	3.5.1.5	Urea hydrolysis	N-cycling	N acquisition
Phosphatase	3.1.3.X	Release of PO_4^-	P-cycling	P acquisition
arylsulfatase	3.1.6.1	Release of SO_4^-	S-cycling	S acquisition

Table 1.2 list of enzymes involved in carbon and other nutrient cycles

1.4 β-glucosidase enzyme in soil

β-glucosidases also known as cellobiase, (EC 3.2.1.21) are glucosidase enzyme which catalyse the hydrolysis of β-glucosidic linkage of various oligosaccharides and glycosides to form glucose and shorter oligosaccharides. They are widely distributed in the living world and playing key roles in soil carbon cycle being involved in degradation of cellulose. Cellulose, a glucose polymer linked by β(1,4)-glucosidic bonds, is the most abundant polysaccharide in the plant residue of terrestrial environment and current understanding shows that soil microorganism have dominant role in the cellulose decomposition (Lynd et al., 2002; Baldrian and Valášková, 2008). Enzymatic hydrolysis of cellulose requires the synergetic action of three different hydrolyzing enzymes; endoglucanase or endo- β-1,4 glucanase (EC 3.2.1.9.1), exoglucanase or exo-cellobiohydrolase (EC 3.2.1.91) and β-1,4 glucosidase or cellobiase (EC 3.2.1.21). Amongst them, β-glucosidase is the rate limiting enzyme (Alef and Nannipieri, 1995) and thus plays a vital role in the global-scale C cycle (Knight and Dick; 2004). Endoglucanase randomly cleaves the β-1,4 glycosidic linkages of cellulose followed by exoglucanase attacks cellulose chain ends to produce the cellobiose. β-glucosidase completes final

step of cellulose hydrolysis by converting cellobiose to simple glucose molecules which is an important C energy source of life to microbes in soil (Esen 1993). Enzymatic reaction of cellulose degradation is shown in figure 1.3.



Fighure 1.3 Sechamatic enzymatic recation of cellulose hydrolysis

1.5 Classification of β-glucosidases

β-glucosidases are a diverse group of hydrolytic enzymes and have been classified according to different criteria. There is no single well defined classification of these versatile enzymes (Singhania et al. 2012). β-glucosidases are categorised as glycoside hydrolases in the IUB Enzyme Nomenclature (1984) based on the type of their catalysing reaction. Glycoside hydrolase enzymes have been assigned the number EC 3.2.1.X, representing their capability to hydrolyse *O*-glycosyl linkages, such as the 1,4- beta-linkage of cellobiose, with the "X" representing the substrate specificity. In the case of β-glucosidases, the full number is EC 3.2.1.21. This defines hydrolysis of terminal, non-reducing beta-D-glucosyl residues with release of beta-D-glucose. Generally β-glucosidases are classified according their substrate specificity (Shewale, 1982; Eyzaguirre et al., 2005) or their Nucleotide Sequence Identity (NSI) (Henrissat and Bairoch, 1996).

Based on substrate specificity, these enzymes have been classified as (1) aryl β-glucosidases, which act on aryl-glucosides, (2) true cellobiases, which hydrolyze cellobiose to release glucose, and (3) broad substrate specificity enzymes, which act on a wide spectrum of substrates. Most of the β-glucosidases characterized so far are placed in the last category. The most accepted classification is

by nucleotide sequence identity scheme, proposed by Henrissat and Bairoch (1996) based on sequence and folding similarities (hydrophobic cluster analysis, HCA) of these enzymes. HCA of a variety of such enzymes suggested that the α-helices and the β-strands were localized in similar positions in the folded conformation (Singhania et al. 2012). According to this method, enzymes with similar and well conserved amino acid sequence motifs are grouped into the same family. This classification can also reflect structural features, evolutionary relationships, and catalytic mechanism of these enzymes. One hundred and fifteen glycoside hydrolase families are listed in the frequently updated Carbohydrate Active enZYme (CAZY) Web site (http://www.cazy.org). The sequence based classification is useful in characterizing the enzymes from the structural point of view (Singhania et al. 2012). Available β-glucosidases mainly fall in glycosyl hydrolase (GH) family 1 and 3 but some β-glucosidases are also found in family 5, 9, 30 and 116. Family 1 includes β-glucosidases from archaebacteria, bacteria, some fungi, plants and mammals whereas Family 3 includes β-glucosidase from bacteria, mold and yeast. Most of family 1 β-glucosidase also show significantly β-galactosidase activity (Cantarel et al., 2009).

1.6 Structure of β-glucosidase

Active sites of all glycoside hydrolases only placed into three general classes, (i) pocket or crater, (ii) cleft or groove, and (iii) tunnel. β-glucosidases enzymes have the pocket or crater topology that is well suited for recognize of a saccharide non-reducing extremity (Davies & Henrissat, 1995), with the depth and shape of the pocket or crater reflecting the number of subsites that contribute to substrate binding and the length of the leaving group (Davies et al., 1997). A number of GH1 β-glucosidase crystal structures have been determined from different organisms, e.g. *Trifolium repens* (clover) (Barrett et al., 1995), *Bacillus polymyxa* (eubacterium) (Sanz-Aparicio et al., 1998), *Bacillus circulans* (Hakulinen et al., 2000), *Zea mays* (maize) (Czjzek et al., 2001), *Thermus nonproteolyticus* (eubacterium) (Wang et al., 2003), *Triticum aestivum* (wheat), *Secale cereale* (rye) (Sue et al., 2006), *Phanerochaete chrysosporium* (white rot fungus) (Nijikken et al., 2007), *Oryza sativa* (rice) (Chuenchor et al., 2008), and *Humicola insolens* (fungi) (de Giuseppe et al., 2014). These studies have helped to understand their mechanism and broad substrate specificity. Comparing to β-glucosidase of GH1 family, β-glucosidase from GH3 family are less well characterized with only a few crystal structures having been solved: β-glucosidase from *Hordeum vulgare* (barley) (Varghese et al., 1999), *Kluyveromyces marxianus* (a yeast), and *Thermotoga neapolitana* (a hyperthermophilic bacterium) (Pozzo et al., 2010).

β-glucosidases from GH1 and GH3 families greatly diverge in their structures like, their sequence identity fold active side residues. β-glucosidases from GH1 are comprised of two conserved carboxylic acid residues on β-strand 4 and 7 (Figure 1.4). These residues act as the catalytic acid/base and nucleophilic, respectively (Henrisaat et al., 1995; Jenkins et al., 1995). On the contrary, GH3 family β-glucosidases contain two domain structures, a $(\beta/\alpha)_8$-barrel followed by an α/β sandwich comprising a 6-starnded β-sheet sandwiched between three α-helices on the other side (Varshese et al., 1999). The catalytic pocket of GH1 β-glucosidases is tight and deep pocket like a narrow tunnel with dead end, whereas GH3 family β-glucosidases contain shallow and open pockets. The structure of GH1 enzymes mainly depends upon substrate conformation compared to GH3 enzymes (Harvey et al., 2000). The functional properties, such as substrate specificity, binding and catalytic mechanism and rate of these enzymes, mainly depend upon structural differences of these enzymes. Henrissat & Davies (1997) proposed convergent evolution to explain the distribution of β-glucosidases in different GH families. In other words, they are adapted by the environment. Structure of β-glucosidases from GH1 and GH3 families are shown in figure 1.4.

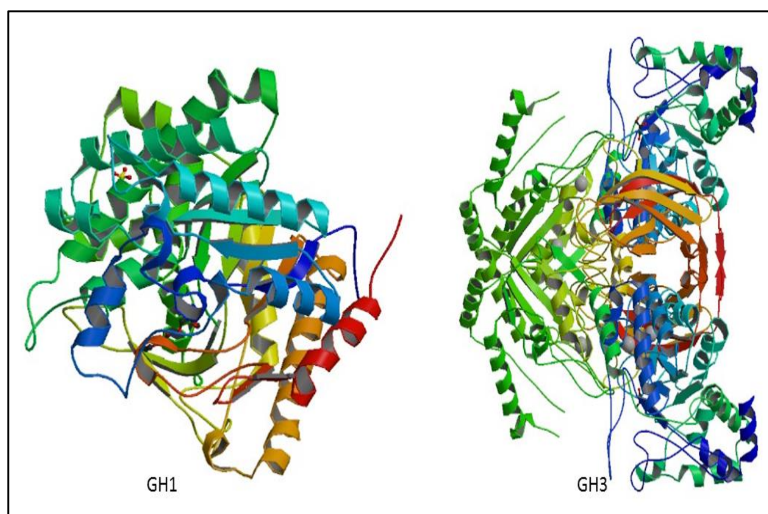


Figure 1.4 Structure of β-glucosidases from GH1 and GH3 families. GH1 β-glucosidases from the fungus *Humicola insolens* from Guiseppe et al (2014) and GH3 β-glucosidases from bacterium *Thermotoga neapolitana* from Pazzo et al (2010). The structural cartoons are coloured in spectrum from blue to red from their N- to C- termini.

Nam et al (2010) have used uncultured soil metagenomes to introduce X-ray crystal structure of β-glucosidase with glucose and cellobiose fragments. They obtained three various active reaction sites of β-glucosidase which are respectively, pre-reaction (Native), intermediate (disaccharide cleavage), and post-reaction (glucose binding) states of the active site pockets (Figure 1.5). These structures present snapshots of catalytic processing of β-glucosidase and intermediate position of crystal

structure gives insights into substrate specificity of β-glucosidase. These structural studies will facilitate the understanding the architectural mechanism responsible for the substrate recognition of β-glucosidase.

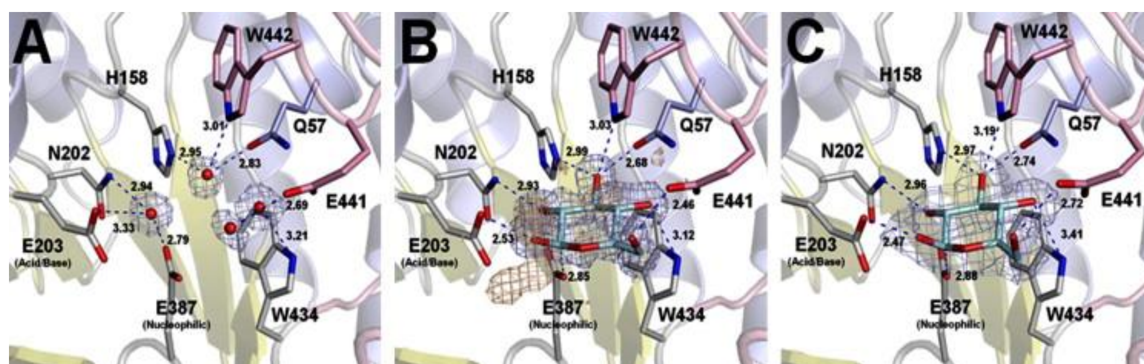


Figure 1.5 Complex structures of the active site pocket in β-glucosidase. Snapshots of the catalytic process of β-glucosidase in the (A) pre-reaction state, (B) intermediate state, and (C) post-reaction state. The electron density of each substrate is shown and was obtained using the final sigma A-weighted 2Fo–Fc electron density map contoured at 1σ (cyan). In the transition state, the electron density map (beige color) is indicated in the Fo–Fc electron density map contoured at 3σ. Broken lines indicate the polar interactions between the enzyme and the substrate. (Nam et al, 2010).

1.7 Origin of β-glucosidase in soil

β-glucosidase can also be extracellular enzymes. They are widely distributed among different organisms. In soil, β-glucosidases are mainly of microbial origin and play an important role in cellulose hydrolysis and induction due to their transglycosylation activities. There are several reports available for β-glucosidase productions from filamentous fungi such *Aspergillus niger* (Gunata and Vallier, 1999), *Aspergillus oryzae* (Riou et al., 1998), *Penicillium brasilianum* (Krogh et al., 2010) *Penicillium decumbens* (Chen et al., 2010), *Phanerochaete chrysosporium* (Tsukada et al., 2006), *Paecilomyces sp.*, (Yang et al., 2009) etc., though there are also various reports of β-glucosidase production from yeasts (majority of them from Candida sp.) and few bacteria. Microscopic fungi are the most important source of β-glucosidase (Singhania et al., 2013).

Echlerová et al (2015) studied 152 fungal strains including one 111 strains of Basidiomycota, 39 of Ascomycota and 2 strains of Mucromycotina. 93 % of species exhibited β-glucosidase activity and production of β-glucosidase was relatively high in fungi. Kenllner et al (2010) also reported that β-glucosidases were normally found in Ascomycota and Basidiomycota. Belmont and Martiny (2013) studied the distribution of 21,985 genes coding proteins related to cellulose degradation in 5,123 sequenced bacterial genomes and confirmed that up to 56% of cellulose degraders have β-

glucosidases. They also reported that β-glucosidase genes coding proteins were presented in almost all bacterium phylum. These facts make both bacteria and fungi suitable tracers for β-glucosidase enzyme production in soil.

1.8 Application of β-glucosidase enzyme

β-glucosidase enzymes play a central role in the degradation of soil organic matter and plant residues. Although no single enzyme activity can deliver a full picture of soil metabolic functioning, β-glucosidase activity has shown to be sensitive to changes in soil and residue management as well as an early indicator of changes in SOC content before these changes are shown by the soil organic C analysis. (Miller and Dick, 1995; Deng and Tabatabai, 1996, Aon and Colaneri, 2001; Turner et al., 2001; Ascota-Martínez et al., 2003; de al Horra et al., 2003; Roldán et al., 2005; Green et al., 2007; Stott et al., 2009). β-glucosidase activity was positively correlated with microbial biomass and reflect the capability of soil to hydrolyse plant residue and dispose nutrients to subsequent crops (Stott et al., 2009). Stott at el (2009) also suggested that β-glucosidase activity might be associated with various soil functions such as, soil biodiversity and habitat, nutrient cycling, filtering and buffering of nutrients and toxic elements and soil physical properties. According to Soil Management Assessment Framework, β-glucosidase activity is sensitive to different managements in various soil types under different climate conditions. For these reasons, its activity is an important soil health indicator (Stott et al., 2009).

β-glucosidases have also enticed significant attention in recent years due to their important roles in diverse biotechnological processes, such as bioethanol production, hydrolysis of isoflavone glucosidase, detoxification of cassava, elimination of bitter components from citrus products etc. (Singhania et al, 2013). Glucose tolerant β-glucosidases can solve the problem of feedback inhibition in bioethanol production and nowadays heat stable β-glucosidases enzymes are used in bioethanol production because these enzymes are capable of performing fast cellulose hydrolysis reaction at elevated temperature, thereby lowering the cost of bioethanol production.

Few species of Aspergilli are known to produce glucose tolerant β-glucosidases. Kim et al (2007) identified two novel β-glucosidases of *Aspergillus fumigatus*. It is expected that more of such glucose tolerant β-glucosidases may be prevalent in nature especially in filamentous fungi. Isolation of such enzymes and knowledge about their properties, sequences and expression patterns can help in designing better enzyme cocktails for biomass hydrolysis as well as in targeted approaches for modifying the glucose tolerance of existing β-glucosidases (Singhania et al., 2013). Molecular studies

on diversity of microbial community with β-glucosidases encoding gene in soil are shown in Table 1.3.

Reference	Aim	Study Site	Methods	Comments
Kellner and Vandenbol (2010)	Identification of transcriptionally expressed fungal genes encoding key lignocellulolytic, chitinolytic and related enzymes	Northern hardwood forest, dominated by *Ascer saccharam* and situated in Oceana County, Michigan, USA	Total RNA extraction followed by cDNA synthesis, degenerate prime designing for functional genes, PCR amplification followed by cloning and sequencing	First paper on diversity of functional fungal encoding genes of enzymes involved Carbon cycle in soil. Lack of enough sequencing depth analysis
Cañizares et al (2011)	Analyses of the molecular diversity and response of β-glucosidase to C stimulation	Microcosm experiment, soils were amended with cellobiose and glucose	Degenerate primer designing for bacterial β-glucosidase, qPCR for quantification of bacterial β-glucosidase genes in soil, PCR followed by cloning and sequencing; protein analysis	First paper on diversity of bacterial β-glucosidase encoding gene in soil and proteomic approach for electrophoretically identifying β-glucosidase activity in gel
Cañizares et al (2012)	Response of soil bacterial structural and functional community under long term management practices in semiarid olive orchards	Semiarid olive orchards, situated in Jaen, south-eastern Spain	Soil DNA and RNA isolation, qPCR, PCR-DGGE fingerprinting	Genomic and transcriptomic approaches were used to gain insight into relationship between soil management and bacterial mediated functions in soil

Cañizares et al (2012)	Response of bacterial community in land management in a high vulnerable and economically vital agroecosystem	Long term field experiment in Jaen, South-eastern, Spain	Use of a complementary biochemical, genomic and transcriptomic methods	First paper to link β-glucosidase activity of soil with detection and expression of bacterial β-glucosidase encoding genes. Use of DGGE for studying community composition instead of high throughput sequencing techniques
Moreno et al (2013)	Study of the main environmental factors; affecting diversity of bacterial β-glucosidase encoding genes in semiarid soils	Semiarid olive orchads field, Jaen, South-eastern Spain	PCR-DGGE fingerprinting followed by cloning and sequencing	First attempt to analyse main environmental factors affecting diversity of bacterial β-glucosidase encoding genes but lack of in depth sequencing analysis
Li et al (2013)	Analyse diversity of microbial community, carrying β-glucosidase genes and CMCase and β-glucosidase activities during composting of cattle manure-rice straw	Horticulture station, Northeast agricultural University of China	Enzyme activity assays, PCR DGGE fingerprinting followed by sequencing of DGGE bands	First paper, comparing both fungal and bacterial communities producing β-glucosidase. Use of low sensitive DGGE fingerprinting and only analysed potential diversity of enzyme encoding genes

Table 1.3 Molecular studies on diversity of microbial community with β-glucosidases encoding gene in soil in chronological order.

1.9 Aims of the PhD research

Understanding organic matter decomposition in the terrestrial ecosystem under different environmental conditions is crucial for estimating global C fluxes and their potential future changes (Štursová et al., 2012; Baldrian et al., 2012). Accumulation of dead plant biomass on soil surface is mostly composed of cellulose, hemicellulose and lignin. Cellulose, a glucose polymer linked by β(1,4)-glucosidic bonds, is the most abundant polysaccharide in the plant residue of terrestrial environment and its degradation was the subject of demanding research for decades, and current understanding shows that soil microorganism have dominant role in this process (Lynd et al., 2002; Baldrian and Valášková, 2008).

Enzymatic hydrolysis of cellulose requires the synergetic action of three different hydrolase enzymes; endoglucanase or endo- β-1,4 glucanase (EC 3.2.1.9.1), exoglucanase or exo-cellobiohydrolase (EC 3.2.1.91) and β-1,4 glucosidase or cellobiase (EC 3.2.1.21). Amongst them, β-glucosidase is rate limiting enzyme (Alef and Nannipieri, 1995) and thus play an important role in the global-scale C cycle (Knight and Dick; 2004). β-glucosidase completes final step of cellulose hydrolysis by converting cellobiose to simple glucose molecules. Owing to its very large microbial diversity, soils are reservoir of C hydrolyzing activities (Nannipieri et al., 2012); however, in spite of their fundamental role in nature, the diversity of microbial β-glucosidase encoding genes is still poorly understood. Many researchers have only focused on measurement of potential β-glucosidase activity in different soils as affected by different biotic and abiotic factors using the present enzyme assays. As shown in Table 1.3, few studies have been carried out to study activity, diversity, abundance and distribution of soil β-glucosidase encoding genes and but only two studies have focused on expression of β-glucosidase encoding genes as detected by mRNA and protein.

Bao et al (2012) obtained several β-glucosidase via metagenomic strategies and several sets of degenerate primers have been designed to analyze β-glucosidase gene diversity in defferent soils (Kellner et al., 2010; Caňizares et al., 2011; Li et al 2013). None of these studies however used an in depth sequencing and only Li et al (2013) studied both fungal and bacterial community using PCR-DGGE approach to analyse only potential diversity of β-glucosidase genes. Baldrian et al (2012) reported that proportion of expressed genes can only analyzed by DNA/RNA ratio. Kellner et al (2010) reported that β-glucosidase genes are normally found in Ascomycota and basidiomycota, whereas Berlemont and Martiny (2013) reported that β-glucosidase genes are present in nearly all bacterium phyla. These facts make both bacteria and fungi suitable tracers involved in enzymatic cellulose hydrolysis and potential carrier of β-glucosidase encoding genes in soil.

The main objective of the project was to discriminate origin of fungal and bacteria β-glucosidases in soil in relation to different soil conditions. The expected benefits from my research project are: *i*) proposition of a new integrated methodology; *ii*) the possible discovery of new β-glucosidases, with potential biotechnological applications, due to the high soil microbial diversity. In fact, the heat stable β-glucosidases are used in bioethanol production because these enzymes are capable of performing fast cellulose hydrolysis reaction at elevated temperature, thereby lowering the cost of bioethanol production; and *iii)* to study the complex soil microflora at functional level to improve understanding of soil quality.

For these reasons, research aims of my thesis were: *i*) to compare enzyme activities with microbial diversity in soils; *ii*) to design new primers for β-glucosidase encoding genes; *iii)* to study diversity of β-glucosidases encoding genes in soil and their expression as mRNA as affected by different environmental and biological conditions; *iv*) to study the phylogenetic distribution of β-glucosidase genes in soil bacteria and fungi.

To fulfill aims of the project, I used both genomic and transcriptomic approaches to detect the presence and determine the expression of β-glucosidase encoding genes in soil under different biological conditions. I have studied rhizosphere effects by using two different varieties of Maize; Lo5 and T250 having high and low nitrogen use efficiency (NUE), respectively. Studies were also carried out on soil of ecological importance; coniferous forest soil under different seasons. Mainly, these studies emphasized to unveil influence of different biological conditions on activity, diversity and expression of β-glucosidase encoding genes and involvement of microbial communities (bacteria and fungi) in C cycle by focusing on cellulose hydrolysis process.

The rhizosphere is an environmental compartment hosting fundamental processes responsible for the ecosystem functioning and crop production (Coleman et al. 1992), including C and nutrient cycling (Helal and Beck 1989), with hosting greater and more active microbial populations than the bulk soil, sustained by the release of root exudates. We hypothesized that maize inbred lines differing for the nitrogen use efficiency (NUE) can affect both composition and activities of soil microbial communities, due to different N uptake and probably different rhizodeposition. Therefore, we studied the changes in the biochemical activity, microbial community structure and diversity of the β-glucosidase genes of the rhizosphere of the maize inbred (*Zea mays* L.) lines Lo5 and T250

characterized by high and low NUE (Balconi et al. 1997; Zamboni et al., 2014), respectively, by using rhizoboxes. We determined cellulase, chitinases, β-glucosidase and β-galactosidase, acid and alkaline phosphomonoesterase, phosphodiesterase, urease and arylsulphatase activities for their important role in C, N and P dynamics of the rhizosphere soil and the microbial community composition using a phylogenetic group specific PCR-DGGE approach in the rhizosphere and bulk soil of both Lo5 and T250 maize lines. Diversity of the β-glucosidase genes was also analyzed using PCR followed by high throughput sequencing using the Illumina Miseq sequencer. Comparative metatranscriptomic was used to study microbial expression profile in the rhizosphere of maize plants differing in their N use efficiencies. Main objectives were to relate the expression of functional genes in the rhizosphere during the growth of two maize lines differing their N use efficiency.

The coniferous forests soils that contains more than one third of all carbon stored on the land. Hence, understanding organic matter decomposition in the coniferous forest ecosystem is crucial for estimating global C fluxes and their potential future changes. This study was performed in two contrasting seasons; late summer when plant photosynthetic activity was at peak and late winter (March) after a prolonged period with no photosynthate input. The aim was to demonstrate how the β-glucosidase genes and transcript pools of bacteria and fungi differ among horizons with different cellulose content and which members of the soil microbial community express the corresponding genes in the two different seasons. Two sets of degenerate primers were designed to amplify fungal β-glucosidase genes of GH1 and GH3 families and two available sets of degenerate primers were used to amplify bacterial β-glucosidase genes of GH1 and GH3 families followed by high throughput sequencing using the Illumina Miseq sequencer.

Chapter 2

"Enzyme activity and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency"

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REGULAR ARTICLE

Enzyme activity and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency

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Abstract
Aims Study of the changes in soil microbial biomass, enzyme activity and the microbial community structure in the rhizosphere of two contrasting maize lines differing in the nitrogen use efficiency (NUE).
Methods The Lo5 and T250 inbred maize characterized by high and low NUE, respectively, were grown in rhizoboxes allowing precise sampling of rhizosphere and bulk soil and solution. We also determined microbial biomass, enzyme activities involved in the C, N, P and S cycles, and the microbial community structure using a phylogenetic group specific PCR-DGGE approach in the rhizosphere and bulk soil of both Lo5 and T250 maize lines.
Results High NUE Lo5 maize induced faster inorganic N depletion in the rhizosphere and larger changes in microbial biomass and enzyme activities than the low NUE T250 maize line. The two maize lines induced differences in the studied microbial groups in the rhizosphere, with the larger modifications induced by the high NUE Lo5 maize line.
Conclusions The Lo5 maize line with higher NUE induced larger changes in soil chemical properties and in the enzyme activity, soil microbial biomass and community structure than the low NUE T250 maize line, probably due to differences in the root exudates of the two maize lines.

Keywords Nitrogen · Maize · Nitrogen use efficiency · Rhizosphere · Microbial activity · Microbial community structure

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Introduction

The rhizosphere has different physico-chemical properties as compared to the bulk soil because of the active or passive release of root exudates, consisting of low molecular weight organic compounds (LMWOCs) such as carboxylic acids, sugars (Hawes et al. 2003) and more complex chemical molecules such as polyphenols (Tomasi et al. 2008), accounting for a significant amount of C fixed by photosynthesis (Uren 2007). Root exudates released by plants for enhancing nutrient uptake, molecular signaling and fixing toxic elements such as Al

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(Uren 2007; Tomasi et al. 2008), sustain larger and more active microbial populations and higher enzyme activity in the rhizosphere than in bulk soil (Gilbert et al. 1999; Badalucco and Nannipieri 2007). Microbial and hydrolase activities in the rhizosphere are more important for plant nutrition and crop production because they can decompose soil organic matter and release inorganic N, P and S which can be taken up by plants (Nannipieri et al. 2012). Differences in root exudates can alter composition of the microbial community in rhizosphere, which depends on plant species, soil properties, plant growth conditions and seasons (Berg and Smalla 2009). The use of culture independent methods has allowed studying both the composition of microbial communities and changes in specific microbial groups (Gomes et al. 2001, 2010). Thus, it has been reported that the α-proteobacteria are most abundant in the rhizosphere (McCaig et al. 1998) while actinomycetes and γ-proteobacteria, involved in decomposition of the soil organic matter (SOM), are more abundant in bulk than rhizosphere soil (Heuer et al. 1997; Ulrich et al. 2008).

Nitrogen is the main nutrient limiting plant growth and crop yield (Raun and Gohnson 1999), and an important goal for the development of sustainable agriculture is to increase plant N use efficiency (NUE). However, plant mechanisms influencing NUE are complex (Xu et al. 2012) and mainly studied using the *Arabidopsis thaliana* model plant in simplified experimental systems, whereas information on microbial biomass, enzyme activity and microbial community structure in the rhizosphere of plants with different NUE is still scarce.

Understanding of the relationship between plant roots and composition of microbial communities of the rhizosphere soil is still a challenge due to the difficulties in precise sampling of this physically restricted, chemically complex and dynamic microenvironment. Studies on the effects of specific root exudates on the composition of soil microbial communities and on the biochemical activity of rhizosphere soil have been carried out using systems mimicking the root exudate release from model root surfaces (Baudoin et al. 2003; Landi et al. 2006; Renella et al. 2007) or by rhizoboxes allowing plant growth and precise sampling of rhizosphere soils (Wenzel et al. 2001; Fitz et al. 2003; Hinsinger et al. 2003; Neumann et al. 2009).

We hypothesized that maize inbred lines differing for the NUE can affect both composition and activities of soil microbial communities, due to different N uptake and probably different rhizodeposition. Therefore, we studied the changes in the biochemical activity and microbial community structure of the rhizosphere of the maize inbred (*Zea mays* L.) lines Lo5 and T250 characterized by high and low NUE (Balconi et al. 1997; Zamboni et al. 2014), respectively, by using rhizoboxes. We determined β-glucosidase, acid and alkaline phosphomonoesterase, phosphodiesterase, urease and arylsulphatase activities for their important role in C, N and P dynamics of the rhizosphere soil.

Materials and methods

Soil properties, maize plants and rhizobox setup

A sandy clay loam soil classified as a Eutric Cambisol (WRB 1998) under conventional maize crop regime, located at Cesa (Tuscany, Central Italy), was sampled from the Ap horizon (0–25 cm). The soil was sieved at field moisture (2 mm), after removing visible plant material. Soil contained 32.1 % sand, 42.2 % silt, 25.7 % clay, 10.8 g kg^{-1} total organic C (TOC), 1.12 g kg^{-1} total N and 6.45 g kg^{-1} total P. After sieving, the soil was immediately used for the rhizobox experiment.

The rhizoboxes consisted of two bulk soil compartments separated by the plant compartment, enclosed by 0.22 μm mesh nylon tissue. Full details about the rhizobox set up are reported by Fitz et al. (2003). Six-hundred g of soil were placed in the two soil compartments immediately before the plantlet insertion in the plant compartment, whereas there was no soil in the plant compartment.

Maize seeds of Lo5 and T250 inbred lines were germinated in Petri dishes containing blotting paper moistened with sterile deionized water. The plantlets were transferred to the plant compartment of rhizoboxes and incubated under the following conditions: 16:8 light/dark period, 200 μE m^{-2} s^{-1} light intensity and temperature of 22/25 °C for the dark and light periods and a relative humidity of 70 %.

The Lo5 and T250 maize lines were grown for 21 and 28 days respectively, which were found, in preliminary experiments, to be a suitable growth period to allow the full colonization of the plant compartment by plant roots. Plants were regularly watered with distilled H_2O because no fertilizers were applied and the inorganic N concentration was also regularly monitored in the rhizosphere by Rhizon® with soil moisture

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samplers so as to prevent plant nutrient starvation due to excessively low inorganic N concentrations, as determined in preliminary studies. The used rhizoboxes allowed precise sampling of rhizosphere due to the presence of fixed sampling groves at precise increment distances from the plant root surface. Soils were analyzed at the end of the experiment and soil samples from Lo5 and T250 maize lines rhizosphere (R) and bulk soil (B) were named as Lo5R, Lo5B, T250R and T250B, respectively.

The soil TOC was determined by wet oxidation with $K_2Cr_2O_7$ according to Walkley and Black (1934), the inorganic N (NH_4^+-N and NO_3^--N) concentration was analyzed by ion selective electrodes (Crison), and the available P extracts was determined according to Olsen and Sommers (1982). All rhizoboxes were prepared with five replicates for each maize line.

Measurement of soil microbial biomass and enzyme activity

Soil microbial biomass was determined by the ATP content according to Ciardi and Nannipieri (1990). Arylesterase activity was determined as described by Zornoza et al. (2009). Acid and alkaline phosphomonoesterase activities were assayed according to Tabatabai and Bremner (1969), and Phosphodiesterase activity as reported by Browman and Tabatabai (1978). β-glucosidase activity was assayed according to Tabatabai (1982). All hydrolase activities were determined at 37 °C for 1 h; after centrifugation at 6,000*g* at 4 °C, the concentration of p-nitrophenol (p-NP) was determined at 400 nm (Lambda 2, Perkin Elmer). Urease activity was determined according to Nannipieri et al. (1974), and the released NH_4^+-N was extracted with 1 M KCl and quantified at 660 nm after reaction with the Nessler reagent. The efficiency of NH_4^+-N recovery, evaluated by standard additions of NH_4^+-N to soil slurries at concentrations in the range of those released by urease and protease activities, was higher than 95 % for all soils.

DNA extraction and PCR-DGGE conditions

The soil DNA was extracted from the five independent replicate rhizoboxes for each maize line by the sequential extraction method described by Ascher et al. (2009) and quantified by a Qubit® 2.0 flurometer (Invitrogen, USA). The molecular weight and fragment length distribution of DNA were checked on 1.5 % agarose gel.

The bacterial specific primers GC-968f and 1401r were used to amplify 16 s rRNA gene fragments (Nübel et al. 1996). A GC rich sequence was attached to the primer 968f (indicating GC-) to prevent complete melting of the PCR products during separation in the denaturing gradient gel. The PCR program was: 90 s at 94 °C, 30 s at 56 °C, 45 s at 72 °C, followed by 33 cycles of 20 s at 95 °C, 30s at 56 °C, 45 s at 72 °C followed by final extension step at 72 °C for 5 min.

The actinomycetes 16 s rRNA gene fragments were amplified with primer set 243f/1401r. The PCR program was according to Heuer et al. (1997): 5 min denaturation at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 63 °C,2 min at 72 °C, followed by final extension step at 72 °C for 10 min. The α and γ proteobacteria 16 s rRNA gene fragments were amplified with primers sets F203α/R1494 and γF383/R1494, respectively. PCR program were according Gomes et al. (2010). For α-proteobacteria: 5 min denaturation at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 56 °C,2 min at 72 °C, followed by final extension step at 72 °C for 10 min. For γ-proteobacteria: 5 min denaturation at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 50 °C,2 min at 72 °C, followed by final extension step at 72 °C for 10 min. A GC clamp was added to all bacterial group specific amplicons by a second PCR amplification with bacterial primers GC-968f/ 1401r (Nübel et al. 1996; Heuer et al. 1997) for prevention of complete melting of the PCR products during separation in the denaturing gradient gel.

Fungal 18 s rRNA gene fragments were amplified using the primer set FF390 and FR1-GC (Vainio and Hantula 2001). A GC rich sequence was attached to the primer FR1-GC (indicating -GC) to prevent complete melting of the PCR products during separation in the denaturing gradient gel. The PCR program was: 8 min denaturation at 94 °C, followed by 30 cycles of 30 s at 94 °C, 45 s at 50 °C,2 min at 72 °C, followed by final extension step at 72 °C for 10 min. The PCR reaction mixture was same for all amplification cycles. Four μl template DNA (40 ng) for first round and 4 μl of PCR product for second round of PCR were amplified with 5U μl^{-1} Dream Taq DNA Polymerase (Thermo Scientific), 10 μM of each primer, 10 mM of each dNTPs, 10X (plus $MgCl_2$ 20 mM) Dream Taq reaction buffer (Thermo Scientific) and 500 $\mu g\ ml^{-1}$ of BSA in final reaction volume of 50 μl. The TProfessional

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Thermocycler (Biometra) was used for all PCR amplifications. All PCR products were assessed on 1.5 % agarose gel to check the correct size of amplicon bands and also quantified by a Qubit® 2.0 flurometer (Invitrogen, USA).

The DGGE analysis was performed using the PhorU System (Ingeny International BV, Netherlands). Briefly, 100 ng of PCR product were loaded on gel. Gel 16S rRNA gene fragment and running conditions for all bacteria groups were: 10 % polyacrylamide gel, urea denaturing gradient 45–65 %, 1X TAE (Tris-acetate-EDTA), 150 V, 60 °C run for 6 h. Gel and running conditions for fungi were: 6 % polyacrylamide gel, denaturing gradient 45–60 %, 1X TAE (Tris-acetate-EDTA), 150 V, 58 °C for 6 h. Gels were stained with SybrGreen (1X) for 45 to 50 min according to Ascher et al. (2009) and analyzed by the GelDoc system image analysis software (Bio-Rad laboratories, USA).

Data analysis

All chemical and biochemical analyses were made from the five independent replicates and the significance of difference between mean values of soil chemical properties, ATP content and enzyme activities was calculated by ANOVA followed by the Fisher PLSD test using the Statview® software (SAS Institute Inc., USA). The DGGE banding patterns were compared with the Quantity-One® software (Bio-Rad Laboratories, USA), with the lanes normalized considering the total signal after background subtraction.. Band positions were converted to Rf values between 0 and 1 and profile similarity was calculated by determining the dice's similarity coefficient (Sørensen 1948) for the total number of lane patterns from the DGGE gel. Calculated similarity coefficients were then used to construct a dendrogram using the unweighted pair group method with arithmetical averages (UPGMA). Rf values and peak intensity data were used to estimate the probability based similarity index (S_{RC}) of Raup and Crick (1979), so as to evaluate similarities within or between soils collected under plant varieties (Moreno et al. 2013). The S_{RC} value is the probability that the randomized similarity would be greater or equal to the observed similarity; S_{RC} values above 0.95 or below 0.05 indicated similarity or dissimilarity (Moreno et al. 2013). The UPGAMA dendrogram, S_{RC} values and similarity clusters analysis were calculated using the PAST (Paleontological Statistic, version 3.X) program. (Hammer et al. 2001). The multivariate relationship between community composition and environmental factors was analyzed by redundancy analysis (RDA) using XLSTAT (ADDINSOFT SARL) software. The Monte Carlo permutation test (500 random permutations) was performed to analyzed significant effect of environmental variables on the observed community composition. Soil chemical and biochemical properties were represented as vectors with length and slope as substantial constraints.

Results

Soil chemical properties

The Lo5 maize line showed a significant faster inorganic N uptake from the rhizosphere solution than the T250 line during the first 2 weeks of growth, as determined by the decrease in the concentration of inorganic N in the rhizosphere solution, which were significantly lower for the Lo5 plant after 4 and 7 days of growth (Fig. 1). Difference in the concentration of inorganic N in the rhizosphere solution were not significant after 10 days and reached similar values after 21 and 28 days (Fig. 1). At the end of the growth period, both plant lines did not alter the rhizosphere and bulk soil pH, whereas the TOC content was significantly higher in the rhizosphere than in the bulk soil of both maize lines (Table 1).

At the end of the growth period, the NH_4^+-N concentrations was significantly lower in the rhizosphere of both the Lo5 and T250 maize lines as compared to their respective bulk soils, whereas the NO_3^--N concentrations were significantly higher in the rhizosphere than in the bulk soil for the T250 maize line but not significantly different for the Lo5 maize line (Table 1). The available P concentrations were significantly higher in the rhizosphere of both the Lo5 and T250 maize lines than in the respective bulk soils, with the highest values in the rhizosphere of the LO5 maize line (Table 1).

Microbial biomass and enzyme activity

The ATP content was significantly higher in the rhizosphere soil than the bulk soil of both the Lo5 and T 250 maize lines, with the highest values in the rhizosphere soil of the Lo5 line (Table 2). With the exception of the arylsulfatase and alkaline phosphomonestrase activites, all measured enzyme activities were significantly higher

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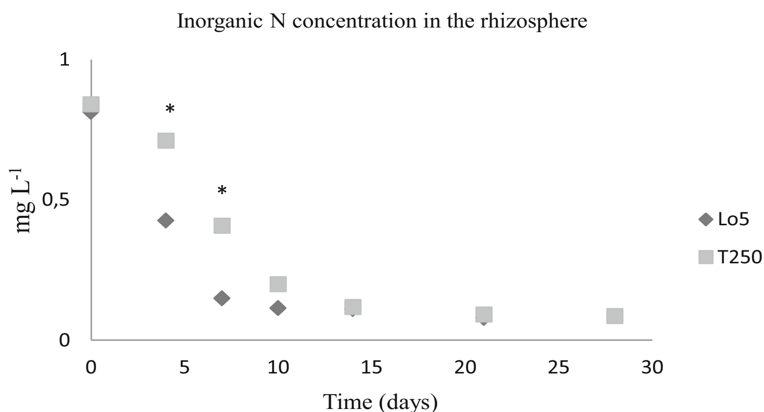

Fig. 1 Inorganic N (NH_4^+-N+ NO_3^--N) in the rhizosphere solution of the Lo5 and T250 maize lines. Symbols * indicate significant differences (P<0.05) between the different maize lines

in the rhizosphere than in the bulk soil of both maize lines. Moreover, the β-glucosidase and urease activities of the Lo5 rhizosphere soil were significantly higher than those of the T 250 rhizosphere soil (Table 2).

Bacterial community structure

The bacterial DGGE profiles revealed that the two maize lines had different effects on the bacterial community in their rhizosphere, as the Lo5R had two additional bands in DGGE profiles compared to the T250R. Dendrogram generated by UPGMA showed that both maize lines clustered separately from each other (Fig. 2) and similarly Lo5 and T250 maize line clustered separately also in Raup and Crick's cluster analysis (Fig. 3). This was confirmed by the S_{RC} values that showed no significant similarity between Lo5R and Lo5B whereas the T250R and T250B were significantly similar (S_{RC}> 0.95) (Table 3). The RDA analysis showed that TOC, NO_3^--N and available P contents and all enzyme activities had strong relationship with bacterial community of Lo5R (Fig. 4). The NH_4^+-N concentration did not have any relationship with the bacterial communities of both rhizosphere and bulk soil of both maize lines, whereas bacterial community of Lo5B, T250R and T250B was not related with any kind of chemical and biochemical variables (Fig. 4).

The DGGE profile of Actinomycetes showed that different shifts occurred in composition of rhizosphere and bulk soil of both maize lines. UPGMA and S_{RC} dendrograms revealed that Lo5 and T250 rhizosphere (Lo5R and T250R) were clustered separately from those of the bulk soil of both maize lines (Figs. 2 and 3). However, the S_{RC} values were not significant for any case and similarity was not greater than expected by chance (0.95>S_{RC}<0.05) (Table 3). The RDA results showed that actinomycetes communities of Lo5R and T250R were related to urease and β-glucosidase activities and composition of actinomycetes of Lo5B was related to the NH_4^+-N concentration (Fig. 4). The TOC, NO_3^--N and available P contents, and arylesterase, arylsufatase, phosphodiesterase, acid and alkaline phosphomonoesterase activities were related to the actinomycetes community structure of bulk soil of T250 maize line (Fig. 4).

The DGGE profile of α-proteobacteria revealed that their community structure differed between the two maize lines and also in both rhizosphere and bulk soil

Table 1 Soil pH values and nutrient availability in the rhizosphere and bulk soil of the two inbred maize lines after the growth period

	$pH_{(H2O)}$	TOC (g kg^{-1})	NH_4^+-N	NO_3^--N (mg kg^{-1})	Available P
Lo5 rhizosphere	7.1^a	13.4^a	0.11^b	0.19^a	22.3^a
Lo5 bulk	7.2^a	9.8^b	0.26^a	0.16^a	12.9^c
T 250 rhizosphere	7.2^a	12.4^a	0.16^b	0.24^a	16.7^b
T 250 bulk	7.2^a	9.8^b	0.22^a	0.20^a	15.1^c

Different superscripts indicate significant differences among mean values in columns

TOC total organic C

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Table 2 ATP content and enzyme activities in the rhizosphere and bulk soil of the two inbred maize lines after the growth period

Soil	ATP (ng kg^{-1})	Arylest.	Ac. Phosph.	Alk. Phosph. (mg p-np kg^{-1}· h^{-1})	Phosphod.	β-gluc.	Arylsulf.	Ure. (mg NH_4^+-N kg^{-1}· h^{-1})
Lo5 rhizosphere	348.3^{a}	270.5^{a}	306.4^{a}	280.8^{a}	162.0^{a}	365.8^{a}	158.7^{a}	61.5^{a}
Lo5 bulk	259.9^{c}	177.5^{b}	222.5^{b}	236.1^{b}	122.2^{b}	169.3^{c}	145.3^{a}	28.3^{c}
T 250 rhizosphere	286.9^{b}	216.3^{a}	290.8^{a}	293.0^{a}	168.1^{a}	193.4^{b}	153.5^{a}	37.1^{b}
T 250 bulk	263.9^{c}	156.7^{b}	208.4^{b}	204.1^{c}	112.7^{b}	140.2^{d}	128.6^{b}	33.3^{c}

Different superscripts indicate significant differences among mean values in columns

Arylest arylesterase activity, *Ac Phosph* acid phosphomonoesterase activity, *Alk Phosph* alkaline phosphonoesterase activity, *Phosphod* phosphodiesterase activity, *β-gluc* β-glucosidase activity, *Arylsulf* arylsulfatase activity, *Ure* urease activity

layers and they clustered separately from each other (Figs. 2 and 3). The S_{RC} values were same (0.5) for α-proteobacteria of all the analyzed soil layers and there is not any significant similarity within or between two maize lines (Table 3). The RDA analysis showed that TOC, NO_3^--N and available P contents, and enzyme activities clustered together with the α-proteobacteria diversity in Lo5R whereas the NH_4^+-N content was related to Lo5B and T250B (Fig. 4), whereas the α-proteobacteria community of T250R showed no relations with the measured soil chemical and biochemical properties (Fig. 4).

The DGGE profile of γ-proteobacteria showed differences between soils of two maize lines. UPGMA and S_{RC} cluster analysis revealed that Lo5 and T250 maize lines clustered separately from each other but there were no differences between rhizosphere and bulk soil of the same maize lines (Figs. 2 and 3). The S_{RC} values showed significant similarity (S_{RC}>0.95) between Lo5R and T250B and between T250R and T250B (Table 3). The RDA results showed that TOC, NO_3^--N and available P contents, and all enzyme activities clustered together with γ-proteobacteria

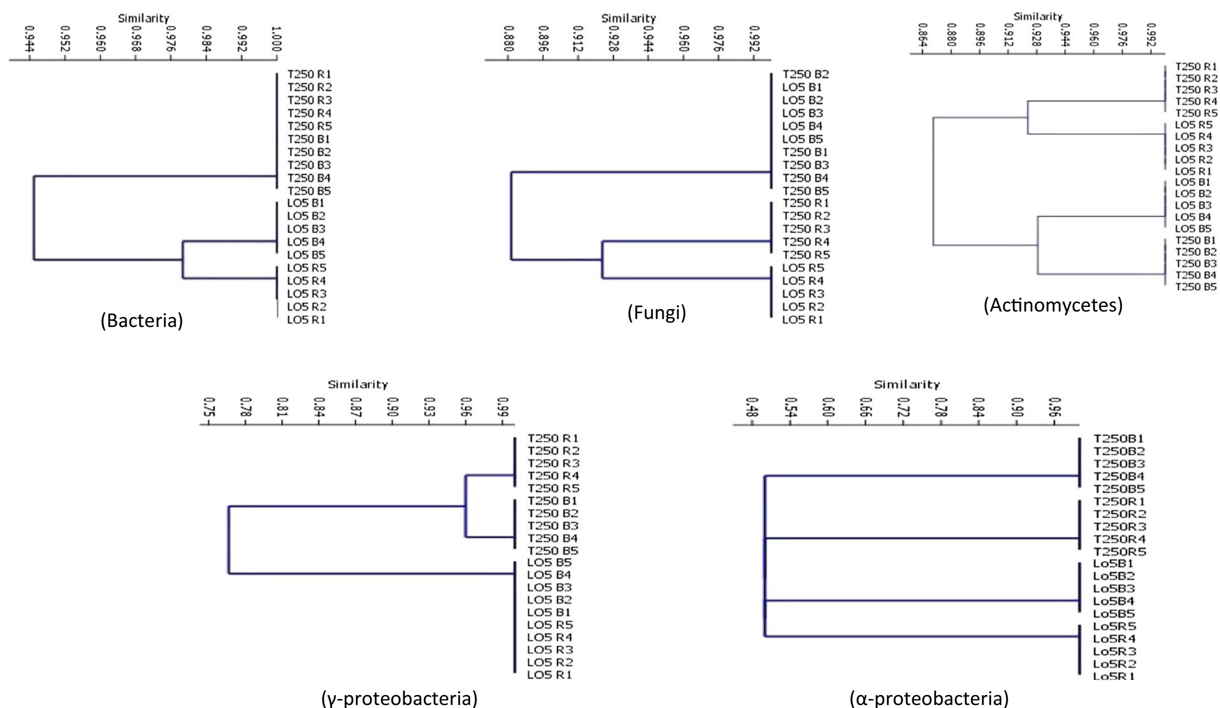

Fig. 2 Dice similarity coefficient based UPGMA dendrogram The letters R and B indicate rhizospher and bulk soil of the Lo5 and T250 maize lines, respectivel

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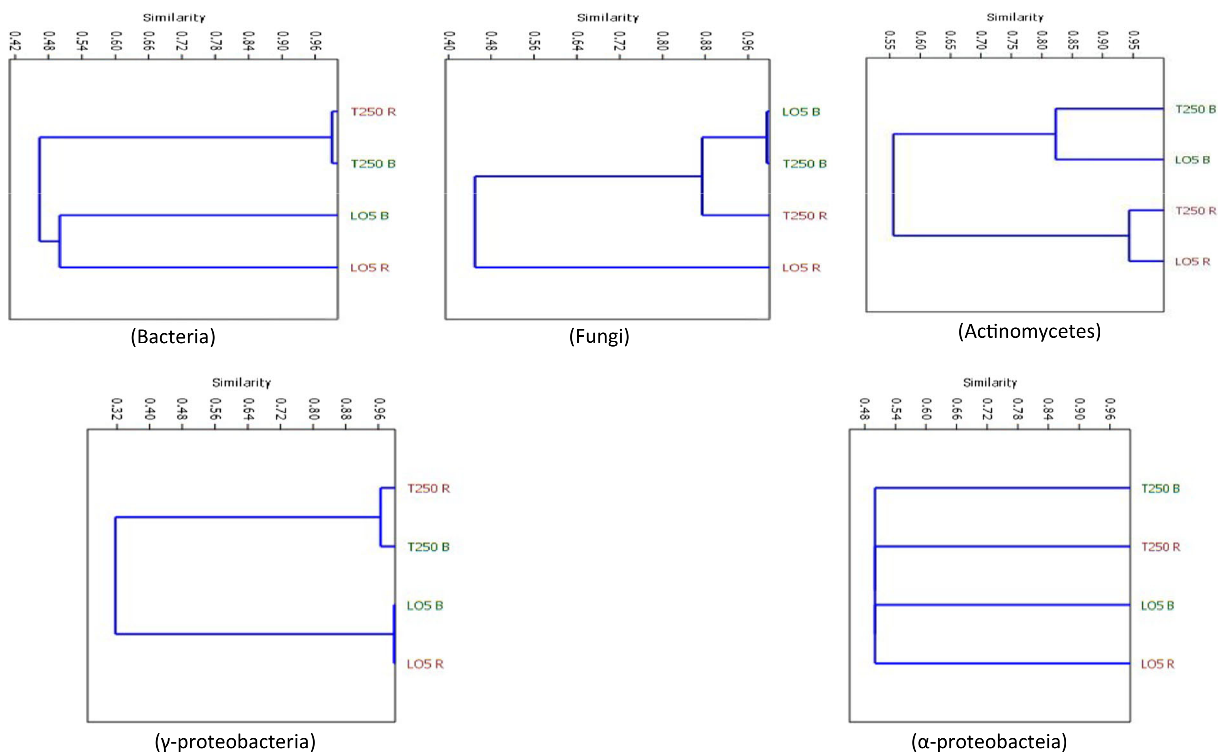

Fig. 3 The S_{RC} cluster analysis. Higher similarity is >0.95 and lower similarity is <0.05. Letters R and B indicate the rhizosphere and bulk soil of the Lo5 and T250 maize lines, respectively

of Lo5R (Fig. 4). The γ-proteobacteria of T250B were related with NH_4^+-N content, whereas γ-proteobacteria T250R and Lo5B were not related to the measured soil chemical and biochemical properties (Fig. 4).

Fungal community structure

The DGGE profile of fungal communities showed that different shifts occurred in composition of rhizosphere soil in both maize lines. The UPGMA and S_{RC} cluster analysis showed that Lo5B and T250B clustered together whereas Lo5R and T250R cluster separately from each other (Figs. 2 and 3). The S_{RC} values indicated significant similarity between Lo5B and T250B (S_{RC} >0.95) but similarity was not identical between Lo5R and T250R (Table 3). The RDA analysis showed that ATP content, β-glucosidase, urease and acid phosphomonoesterase activities were related with fungal diversity of Lo5R whereas NH_4^+-N content was related to the Lo5B (Fig. 4). The T250R and T250B were not related to the measured soil chemical and biochemical properties (Fig. 4). The TOC, NO_3^--N and available P contents and other enzyme activities clustered together and were not related to fungal community of any soil (Fig. 4).

Discussion

The two maize inbred lines showed a different N uptake capacity resulting in a significantly faster depletion of the inorganic N pool in the Lo5 than in T250 rhizosphere (Fig. 1). This result confirmed those of Balconi et al. (1997) who reported that in field experiments the Lo5 adsorbed higher N amounts than the T250 line, likely related to the higher N acquisition capacity of the Lo5 as compared to the T 250 line. Locci et al. (2001) and Zamboni et al. (2014) reported that the induction times of NO_3^--N uptake were faster for the Lo5 than for T250 maize line when seedlings were exposed to NO_3^--N.

The two maize lines also significantly increased the TOC concentrations and inorganic P availability in the rhizosphere soil, with the highest values found for the Lo5 line (Table 1). The increase of dissolved C was likely due to the release of root exudates during the plant

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Table 3 Probability based similarity index (S_{RC}) values of the Raup and Crick probability analysis

Soil		Bacteria	
	Lo5 R	Lo5 B	T250 R
Lo5 B	0.500		
T250 R	0.410	0.500	
T250 B	0.436	0.500	**0.989**
		Actinomycetes	
	Lo5 R	Lo5 B	T250 R
Lo5 B	0.285		
T250 R	0.937	0.363	
T250 B	0.875	0.823	0.698
		α-proteobacteria	
	Lo5 R	Lo5 B	T250 R
Lo5 B	0.500		
T250 R	0.500	0.500	
T250 B	0.500	0.500	0.500
		γ-proteobacteria	
	Lo5 R	Lo5 B	T250 R
Lo5 B	**0.999**		
T250 R	0.462	0.453	
T250 B	0.170	0.173	**0.961**
		Fungi	
	Lo5 R	Lo5 B	T250 R
Lo5 B	0.281		
T250 R	0.783	0.870	
T250 B	0.285	**0.996**	0.850

The letters R and B indicate the rhizosphere and bulk soil, respectively. Values in bold represent significant similarity ($P>0.95$) between microbial communities

growth, whereas the higher P availability can be ascribed to the P solubilization by the root exudates and rhizosphere microorganisms, including mineralization of soil organic P by phosphomonoesterases (Chhabra et al. 2013; Nannipieri et al. 2011). It is also possible that the higher P availability in the rhizosphere of both Lo5 and T250 maize lines can be related to the release of P from inorganic pools by phenolic compounds released from roots which are capable to solubilizing P from inorganic pools (Tomasi et al. 2008; Cesco et al. 2012). Future work is needed to examine the root exudation profile of the two inbred maize lines and to detect the presence of molecules with high P solubilization capacity.

The highest TOC concentration in the Lo5 rhizosphere may depends on a greater release of root exudates by this maize line as part of the anion balance strategy of this plant. In fact, nitrate uptake by plants needs a counterbalance of the excessive anion with release of OH^-, HCO_3^- anions and low molecular weight organic acids (LMWOAs) in the rhizosphere (Hinsinger et al. 2003). Release of the root exudates also likely enhanced the microbial biomass in the rhizosphere of the two maize lines as compared to bulk soil (Table 2). The release of root exudates is generally sufficient to remove the C limitation to microbial activity in soil (Glanville et al. 2012) and the decomposition of root exudates likely contributed to the increase in the ATP content and enzyme activities of the rhizosphere soil (Table 1). According to Pausch et al. (2013) maize rhizodepositions are rapidly mineralized by the rhizosphere microorganisms and partially incorporated into rhizosphere microbial biomass and TOC, and LMWOCs support microbial growth in the rhizosphere (Renella et al. 2007) in the rhizosphere.

The significant stimulation of hydrolase activities involved in the C, N and P solubilization indicated that the synthesis and release of microbial extracellular enzymes involved in SOM decomposition contributed to the plant acquisition of nutrients (Schimel and Bennett 2004; Nannipieri et al. 2011). The relation between the release of root exudates, faster C and N turnover and release of extracellular enzymes by microorganisms in the rhizosphere has been also reported (Badalucco and Nannipieri 2007; Phillips et al. 2011). The increase in β-glucosidase and arylesterase activities, which hydrolyse, respectively, cellobiose and aromatic esters can also depend on the stimulation of microbial enzyme synthesis upon the release of glycosides and aromatic esters in the rhizosphere (Basu et al. 1999). However, plant roots can release various hydrolytic and oxo-reductase enzymes including β-1,3-glucanases, chitinases, proteases and phosphohydrolases for controlling pathogens, attracting plant beneficial microorganisms and solubilizing nutrients in the rhizosphere (Bais et al. 2004; Tomscha et al. 2004; Basu et al. 2006; Badalucco and Nannipieri 2007; Bressan et al. 2009). Synthesis of hydrolytic enzymes by soil microorganisms after the release of LMWOCs has been reported in model rhizosphere studies (Renella et al. 2007). However, proteomic studies on the root exudation profiles are needed in rhizobox studies to better understand the origin of the increased enzyme activity in the rhizosphere of the two studied maize lines (Nannipieri et al. 2012).

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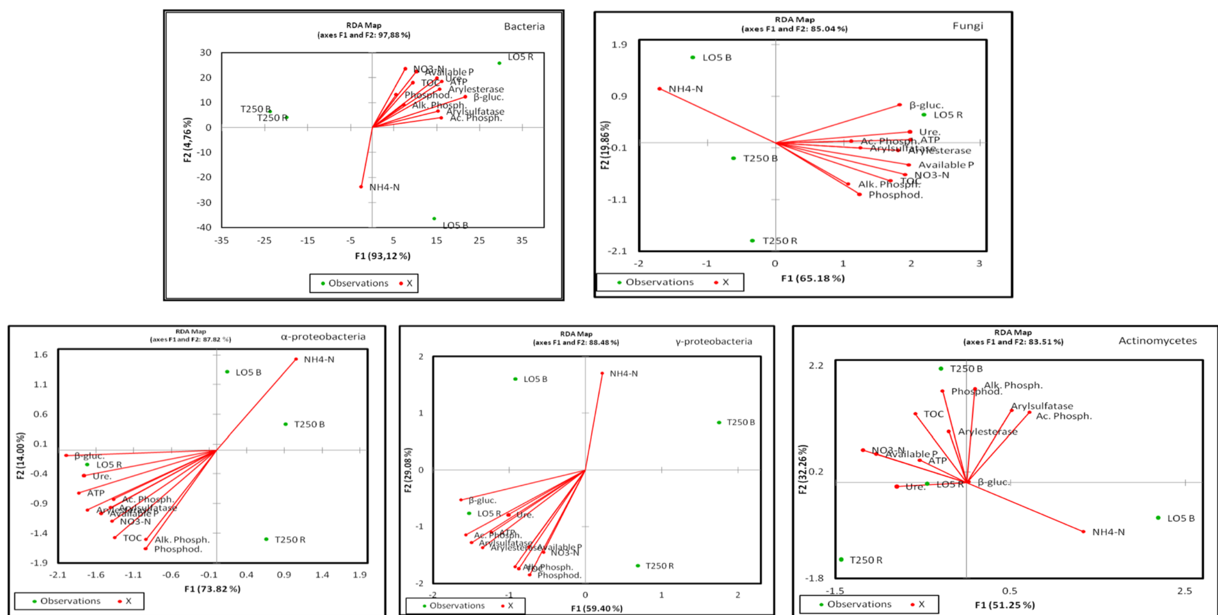

Fig. 4 Redundancy analysis (RDA) plots of DGGE band patterns and environmental variables for the Lo5 and T250 maize lines. Letters R and B indicate the rhizosphere and bulk soil of the Lo5 and T250 maize lines, respectively. Legend: Arylest=arylesterase activity; Ac Phosph=acid phosphomonoesterase activity; Alk Phosph=alkaline phosphonoesterase activity; Phosphod=phosphodiesterase activity; β-gluc=β-glucosidase activity; Arylsul=arylsulfatase activity; Ure=urease activity. Soil chemical and biochemical properties were represented as vectors. Letters 'T' indicates the DGGE band numbers

Several biotic and abiotic factors influence the microbial community structure. Plants induce changes in the soil microbial populations mainly by the release of root exudates, which may be different for different plant genotype, selecting specific microbial populations in the rhizosphere (Berg and Smalla 2009; Sanguin et al. 2006). In our study, Lo5 maize line showed higher N uptake efficiency than the T250 maize line from rhizosphere soil, and this faster N depletion in rhizosphere could likely cause greater changes in the microbial community structure and functions in rhizosphere of the two maize lines than the bulk soil. Changes in the composition of the rhizosphere microbial communities of both maize lines were observed, in agreement with previous studies (Smalla et al. 2001). Selection of specific microbial groups in the rhizosphere of different maize cultivars has been reported by Miller et al. (1989). Our results were in agreement with those of García-Salamanca et al. (2012) who showed that abundance of actinomycetes of both rhizosphere and bulk soil depended on the maize cultivar. In general, it is well established that the selection of bacterial phylogenetic groups depends on the plant cultivar and soil type, but also plant development stage, with more changes induced by young than old plant roots (Gomes et al. 2001; Berg and Smalla 2009). The RDA analysis indicated that proteobacterial community composition in the Lo5 rhizosphere was mainly related to the soil chemical properties (except NH_4^+-N content) and to soil enzyme activity whereas the Actinomycetes community composition was mainly related to urease and β-glucosidase activities (Fig. 4). Probably synthesis of the measured enzyme activities occurred in all proteobacteria, whereas synthesis of β-glucosidase and urease prevailed over the other measured enzymes in actinomycetes and fungi. However, further soil proteogenomic studies for detecting enzyme proteins and enzymes encoding genes (Nannipieri et al. 2012) is needed to confirm this hypothesis.

Our results showed that NH_4^+-N content often clustered separately from the studied microbiological and biochemical soil parameters of the rhizosphere and bulk soil of the two maize lines. Previous work has reported differences in the diversity of microbial groups involved in N turnover in the rhizosphere (Briones et al. 2002; Bremer et al. 2007). The role of NH_4^+-N in plant NUE as well as the diversity of genes involved in N turnover (e.g. *nif*H, *nir*K, *nir*S, *nos*Z and *amo*A) in the rhizosphere of plants with different NUE deserve future research to further understand the importance of these factors in global plant NUE.

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The two maize lines induced shifts in the fungal communities composition (Table 3) confirming the previous findings by Broeckling et al. (2007). The fungal community composition of the Lo5 rhizosphere soil was also related to β-glucosidase, acid phosphomonoestarase and urease activities, whereas no relationships were observed between chemical properties and enzyme activity and the fungal community composition of the T250 maize line. However, further studies to better understanding of which factors are responsible for the link between the fungal community structure and β-glucosidase, acid phosphomonoestarase and urease activity in the rhizosphere are urgently needed

Although the used rhizoboxes allowed precise sampling of rhizosphere, we observed differences in alkaline phosphatase, β-glucosiadase and arylsulfatase activities and some microbial groups (e.g. bacteria and γ-proteobacteria) in Lo5 and T250 bulk soil; this could be due to diffusion of enzymes and movement of some microbial species from the rhizosphere towards the bulk soil. The relative small differences between the bacteria and γ-proteobacteria community structure of the rhizosphere as compared to bulk could be due to the low sensitivity of the PCR-DGGE technique we used, and probably better characterization of the microbial the microbial diversity can be achieved by sequencing.

The NUE has been generally defined on the ability of a genotype to acquire nutrients and use them for biomass production (Blair 1993). Overall, our results indicate that while plant genetic and physiological mechanisms are of fundamental importance in their nutrient acquisition (Fig. 1), they also show that maize the line with higher NUE also enhanced microbial activity in the rhizosphere; therefore both plant and environmental factors likely concur the higher global plant NUE and should be maintained to pursuit best management practices in the agro-ecosystems.

Conclusions

The adopted experimental set up allowed to study the changes induced in chemical parameters, enzyme activity, microbial biomass and microbial community structure in the rhizosphere of Lo5 and T 250 inbred maize lines characterized by high and lower NUE, respectively. Overall, our results indicate that while plant genetic and physiological mechanisms are of fundamental importance in their nutrient acquisition (Fig. 1), they also show that maize lines with higher NUE also enhance microbial activity in the rhizosphere; therefore both factors likely concur the higher global plant NUE and should be maintained to pursuit best management practices in the agro-ecosystems. Future research should investigate the diversity of microbial functional groups involved in N Turnover in the rhizosphere of plants with different NUE to identify eventual selection of specific biochemical mechanisms.

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References

Ascher J, Ceccherini MT, Pantani OL, Agnelli A, Borgogni F, Guerri G, Nannipieri P, Pietramellara G (2009) Sequential extraction and fingerprinting of soil metagenome. Appl Soil Ecol 42:176–181

Badalucco L, Nannipieri P (2007) Nutrient transformation in the rhizosphere. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere Biochemistry and organic substances at the soil-plant interface. CRC Press, Boca Raton, pp 111–133

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26–32

Balconi C, Brosio D, Motto M (1997) Analysis of nitrogen partitioning in maize. Maize Gen Coop News 71:10–11

Basu U, Francis JL, Whittal RM, Stephens JL, Wang Y, Zaiane OR, Goebel R, Muench DG, Good AG, Taylor GJ (2006) Extracellular proteomes of *Arabidopsis thaliana* and *Brassica napus* roots: analysis and comparison by MudPIT and LC-MS/MS. Plant Soil 286:357–376

Baudoin E, Benizri E, Guckert A (2003) Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biol Biochem 35:1183–1192

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Blair G (1993) Nutrient efficiency - what do we really mean. In: Genetic Aspects of Plant Mineral Nutrition Randall PJ, Delhaize E, Richards RA, Munns R, (Eds.) Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 205–213

Bremer C, Braker G, Matthies D, Reuter A, Engels C, Conrad R (2007) Impact of plant functional group, plant species and sampling time on the composition of *nir*K-type denitifier communities in soil. Appl Environ Micrbiol 73:6876–6884

Author's personal copy

Bressan M, Roncato MA, Bellvert F, Comte G, Haichar FZ, Achouak W, Berge O (2009) Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. ISME J 3:1243–1257

Briones AM, Okabe S, Umemiya Y, Ramsing N, Reichardt W, Okuyama H (2002) Influence of different cultivars on populations of ammonia-oxidizing bacteria in the root environment of rice. Appl Environ Microbiol 68:3067–3075

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2007) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738–744

Browman MG, Tabatabai MA (1978) Phosphodiesterase activity of soils. Soil Sci Soc Am J 42:284–290

Cesco S, Mimmo T, Tonon G, Tomasi N, Pinton R, Terzano R, Neumann G, Weisskopf L, Renella G, Landi L, Nannipieri P (2012) Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. Biol Fertil Soils 48:123–149

Chhabra S, Brazil D, Morrissey J, Burke JI, O'Gara FN, Dowling D (2013) Characterization of mineral phosphate solubilization traits from a barley rhizosphere soil functional metagenome. Microbiol Open 2:717–724

Ciardi C, Nannipieri P (1990) A comparison of methods for measuring ATP in soil. Soil Biol Biochem 22:725–727

Fitz WJ, Wenzel WW, Zhang H, Nurmi J, Štípek K, Fischerová Z, Schweiger P, Köllensperger G, Ma LQ, Stingeder G (2003) Rhizosphere characteristics of the arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval efficiency. Environ Sci Technol 37:5008–5014

García-Salamanca A, Molina-Henares AM, Dillewijn PV, Solano J, Pizarro-Tobías Roca A, Duque E, Ramos JL (2012) Bacterial diversity of rhizosphere of maize and surrounding carbonate-rich bulk soil. Microb Biotechnol 6:34–44

Gilbert GA, Knight JD, Vance CP, Allan DL (1999) Acid phosphatase activity in phosphorus-deficient white lupin roots. Plant Cell Environ 22:801–810

Glanville H, Rousk J, Golyshin P, Jones DL (2012) Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. Soil Biol Biochem 48:88–95

Gomes NCM, Heuer H, Schonfield J, Costa R, Hagler LM, Smalla K (2001) Bactreial diversity of rhizosphere of maize (*Zea mays*) grown in tropical soil studies by temperature gradient gel electrophoresis. Plant Soil 232:167–180

Gomes NCM, Landi L, Smalla K, Nannipieri P, Brookes PC, Renella G (2010) Effect of Cd- and Zn- enriched sewage sludge on soil bacterial and fungal communities. Ecotoxicol Environ Saf 73:1255–1263

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistic software package for education and data analysis. Palaeontol Electron 4:1–9

Hawes MC, Bengough G, Cassab G, Ponce G (2003) Root caps and rhizosphere. J Plant Growth Regul 21:352–367

Heuer H, Krsek M, Baker P, Smalla K, Wellington EMH (1997) Analysis of actinomycete communities by specific amplification of genes encoding 16 s rRNA and gel electrophoretic saparation in denaturing gradients. Appl Environ Microbiol 63:3233–3241

Hinsinger P, Plassard C, Tang CX, Jaillard B (2003) Origins of root mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248:43–59

Landi L, Valori F, Ascher J, Renella G, Falchini L, Nannipieri P (2006) Root exudate effects on the bacterial communities CO2 evolution nitrogen transformations and ATP content of rhizosphere and bulk soils. Soil Biol Biochem 38:509–516

Locci G, Santi S, Monte R, Pinton R, Varanini Z (2001) Involvement of plasma membrane H^+-ATPase in nitrate uptake by maize genotypes. In: Horst W, Schenk MK, Bürkert A, Claassen N, Flessa H, Frommer WB, Goldbach HE, Olfs HW, Römheld V, Sattelmacher B, Schmidhalter U, Schubert S, von Wirén N, Wittenmayer L (eds) Plant nutrition-Food security and sustainability of agro-ecosystems. Kluwer Academic Publishers, Dordrecht, pp 184–185

McCaig AE, Glover LA, Prosser TI (1998) Molecular analysis of bacterial community structure under different land management regimes by using 16 s ribosomal DNA sequence data and denaturing gradient gel electrophoresis banding patterns. Appl Environ Microbiol 67:4554–4559

Miller HJ, Henken G, van Veen JA (1989) Variation and composition of bacterial populations in the rhizosphere of maize, wheat, and grass cultivars. Can J Microbiol 35:656–660

Moreno B, Canizares R, Nuňez R, Benitez E (2013) Genetic diversity of bacterial β-glucosidase encoding genes as a function of soil management. Boil Fertil Soil 49:735–745

Nannipieri P, Ceccanti B, Cervelli S, Sequi P (1974) Use of 01 M pyrophosphate to extract urease from a podzol. Soil Biol Biochem 6:359–362

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In Bunemann EK, et al. (Eds) Phosphorus in Action Soil Biology 26 Springer Verlag Berlin Heidelberg pp 215–241

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Fornasier F, Moscatelli MC, Marinari S (2012) Soil enzymology: clasical and molecular approches. Biol Fertil Soil 48:743–762

Neumann G, George TS, Plassard C (2009) Strategies and methods for studying the rhizosphere the plant science toolbox. Plant Soil 321:431–456

Nübel U, Engelen B, Felske A, Anaidr J, Wieshber A, Amann RI, Ludwig W, Backhaus H (1996) Sequence heterogieties of genes encoding 16 s rRNA in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis. J Bacteriol 178:5636–5643

Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Miller RH, Keeney DR (Eds) Methods of soil analysis 2nd edition Part 2 ASA-SSSA Madison WI USA pp 403–430

Pausch J, Tian J, Riederer M, Kuzyakov Y (2013) Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study. Plant Soil 364:273–285

Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol Lett 14:187–194

Raun W, Gohnson GV (1999) Improving nitrogen use efficiency for cereal production. Agron J 91:357–363

Raup DM, Crick RE (1979) Measurment of faunal similarity in paleontology. J Paleontol 53:1213–1227

Renella G, Landi L, Valori F, Nannipieri P (2007) Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clayey and a sandy soil. Appl Soil Ecol 36:124–129

Sanguin H, Remenant B, Dechesne A, Thioulouse J, Vogel TM, Nesme X, Moënne-Loccoz Y, Grundmann GL (2006)

Author's personal copy

Potential of a 16S rRNA-based taxonomic microarray for analyzing the rhizosphere effects of maize on *Agrobacterium* spp. and bacterial communities. Appl Environ Microbiol 72:4302–4312

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Smalla K, Wieland G, Buchner A, Zock A, Parry J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: Plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67:4742–4751

Sørensen T (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. Biologiske Skrifter 5:1–34

Tabatabai MA (1982) Soil enzymes. In: Page AL, Miller RH, Keeney DR (Eds) Methods of Soil Analysis Part 2 Chemical and Microbiological Properties 2nd Ed American Society of Agronomy/Soil Science Society of America Madison WI pp 903–947

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1:301–307

Tomasi N, Weisskopf L, Renella G, Landi L, Pinton R, Varanini Z, Nannipieri P, Torrent J, Martinoia E, Cesco S (2008) Flavonoids of white lupin roots participate in phosphorus mobilization from soil. Soil Biol Biochem 40:1971–1974

Tomscha JL, Trull MC, Deikman J, Lynch JP, Guiltinan MJ (2004) Phosphatase under-producer mutants have altered phosphorus relations. Plant Physiol 135:334–345

Ulrich A, Klimke G, Wirth S (2008) Diversity and activity of cellulose decomposting bacteria isolated from a sandy and loamy soil after long term manure application. Microb Ecol 55:512–522

Uren NC (2007) Types amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R Varanini Z Nannipieri P (Eds) The Rhizosphere Biochemistry and Organic Substances at the Soil-Plant Interface Taylor and Francis Group Boca Raton FL pp 1–21

Vainio EJ, Hantula J (2001) Genetic differntation between European and North American population of *Phlebiopsis gigantean*. Micologia 92:436–446

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci 63:251–263

Wenzel WW, Wieshammer G, Fitz WJ, Puschenreiter M (2001) Novel rhizobox design to assess rhizosphere characteristics at high spatial resolution. Plant Soil 237:37–45

WRB World Reference Base for Soil Resources (1998) World Soil Resources Reports 84 FAO Rome

Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63:153–182

Zamboni A, Astolfi S, Zuchi S, Pii Y, Guardini K, Tononi P, Varanini Z (2014) Nitrate induction triggers different transcriptional changes in a high and a low nitrogen use efficiency maize inbred line. J Integr Plant Biol. doi:10.1111/jipb.12214

Zornoza R, Landi L, Nannipieri P, Renella G (2009) A protocol for the assay of arylesterase activity in soil. Soil Biol Biochem 41:659–662

Table S-1. Probability based similarity index (S_{RC}) values of the Raup and Crick probability analysis with all replicates. The letters R and B indicate the rhizosphere and bulk soil, respectively. Values in bold represent significant similarity ($P > 0.95$) between microbial communities.

Bacteria	**L05 R1**	**L05 R2**	**L05 R3**	**L05 R4**	**L05 R5**	**L05 B1**	**L05 B2**	**L0 5B3**	**L05 B4**	**L05 B5**	**T250 R1**	**T250 R2**	**T250 R3**	**T250 R4**	**T250 R5**	**T250 B1**	**T250 B2**	**T250 B3**	**T250 B4**
L05R2	0.96																		
L05R3	0.96	0.96			0														
L05R4	0.96	0.98	0.97																
L05R5	0.96	0.96	0.96	0.96															
L05B1	0.46	0.46	0.46	0.46	0.46														
L05B2	0.47	0.46	0.46	0.46	0.46	0.96													
L05B3	0.46	0.46	0.47	0.46	0.46	0.96	0.97	1											
L05B4	0.45	0.46	0.47	0.46	0.46	0.97	0.96	0.97											
L05B5	0.46	0.46	0.46	0.46	0.46	0.96	0.96	0.96	0.96										
T250R1	0.44	0.43	0.43	0.43	0.44	0.45	0.43	0.44	0.43	0.44									
T250R2	0.43	0.44	0.44	0.44	0.43	0.44	0.43	0.43	0.44	0.43	1.00								
T250R3	0.45	0.44	0.43	0.44	0.43	0.43	0.43	0.44	0.43	0.44	0.99	1.00							
T250R4	0.44	0.44	0.44	0.43	0.43	0.44	0.43	0.44	0.44	0.44	1.00	1.00	1.00						
T250R5	0.43	0.43	0.44	0.43	0.44	0.43	0.44	0.44	0.43	0.44	1.00	1.00	1.00	1.00					
T250B1	0.43	0.44	0.43	0.43	0.43	0.44	0.43	0.43	0.43	0.44	0.99	1.00	1.00	1.00	0.99				
T250B2	0.43	0.43	0.44	0.44	0.44	0.44	0.44	0.44	0.43	0.44	1.00	1.00	1.00	1.00	1.00	0.99			
T250B3	0.43	0.44	0.43	0.43	0.44	0.43	0.44	0.43	0.43	0.44	1.00	1.00	1.00	0.99	1.00	1.00	1.00		
T250B4	0.43	0.44	0.43	0.43	0.44	0.44	0.43	0.44	0.44	0.44	0.99	0.99	1.00	1.00	0.99	1.00	1.00	0.99	
T250B5	0.43	0.44	0.44	0.44	0.43	0.43	0.43	0.44	0.44	0.44	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

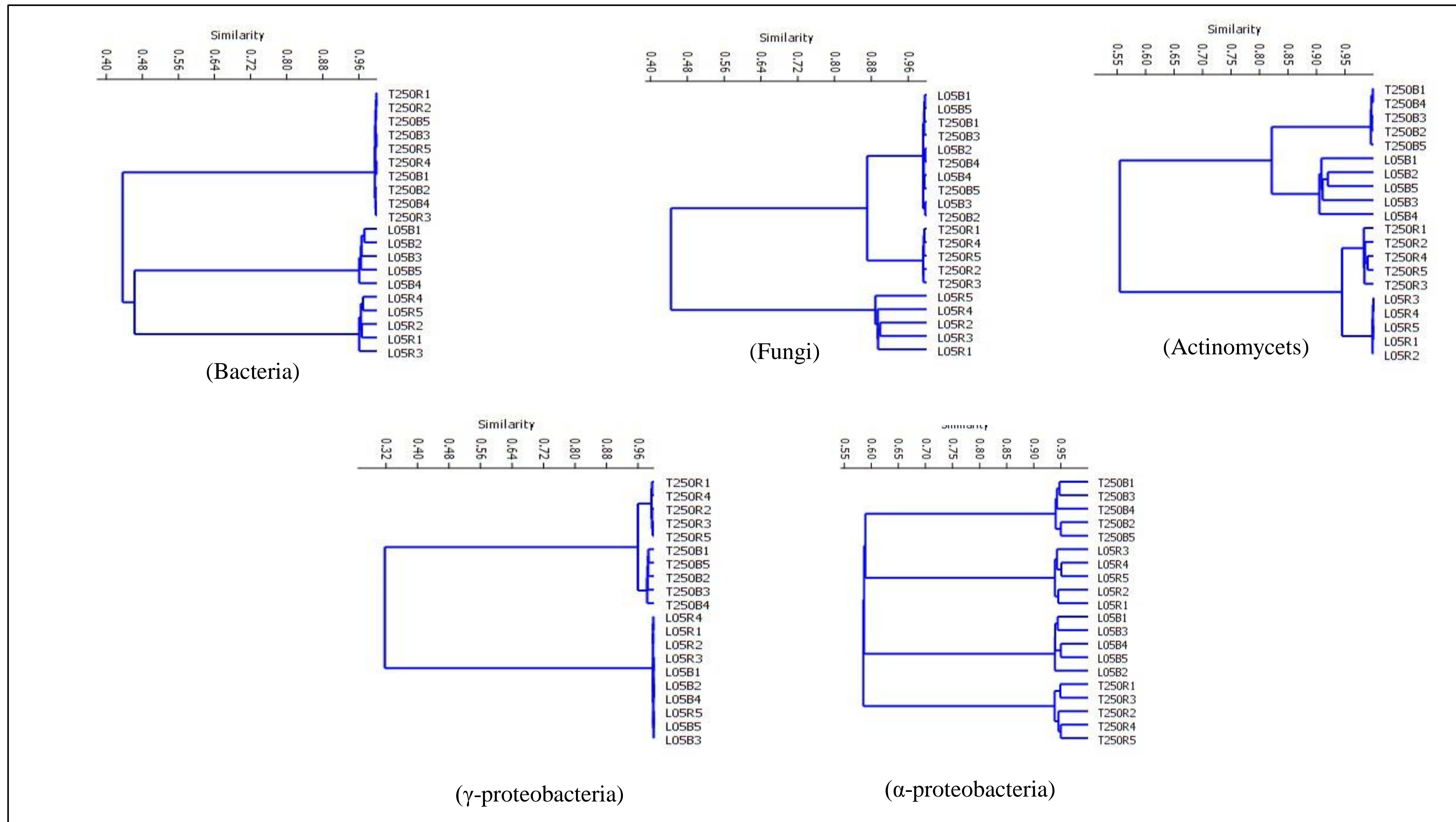
Fungi	**L05 R1**	**L05 R2**	**L05 R3**	**L05 R4**	**L05 R5**	**L05 B1**	**L05 B2**	**L0 5B3**	**L05 B4**	**L05 B5**	**T250 R1**	**T250 R2**	**T250 R3**	**T250 R4**	**T250 R5**	**T250 B1**	**T250 B2**	**T250 B3**	**T250 B4**
L05R2	0.89																		
L05R3	0.88	0.88																	
L05R4	0.89	0.89	0.89																
L05R5	0.90	0.89	0.89	0.90															
L05B1	0.28	0.27	0.28	0.29	0.28														
L05B2	0.29	0.26	0.28	0.27	0.27	0.99													
L05B3	0.29	0.28	0.29	0.29	0.28	0.99	0.99												
L05B4	0.28	0.30	0.27	0.27	0.27	0.99	1.00	0.99											
L05B5	0.27	0.28	0.28	0.28	0.27	0.99	0.99	0.99	0.99										
T250R1	0.78	0.77	0.77	0.77	0.78	0.88	0.86	0.88	0.88	0.88									
T250R2	0.79	0.77	0.78	0.78	0.78	0.88	0.86	0.87	0.88	0.88	0.99								
T250R3	0.77	0.79	0.78	0.77	0.78	0.88	0.87	0.87	0.87	0.85	0.99	0.99							
T250R4	0.77	0.78	0.78	0.77	0.78	0.87	0.87	0.87	0.87	0.88	0.99	0.99	0.99						
T250R5	0.78	0.76	0.78	0.77	0.78	0.88	0.87	0.87	0.87	0.86	0.99	0.99	0.99	0.99					
T250B1	0.26	0.27	0.28	0.29	0.28	0.99	0.99	0.99	0.99	0.99	0.88	0.88	0.88	0.88	0.87				
T250B2	0.29	0.29	0.28	0.28	0.29	0.99	0.99	0.99	0.99	0.99	0.88	0.87	0.87	0.86	0.86	0.99			
T250B3	0.28	0.28	0.28	0.28	0.27	0.99	0.99	0.99	0.99	0.99	0.87	0.86	0.89	0.87	0.87	0.99	0.99		
T250B4	0.28	0.28	0.28	0.28	0.28	1.00	0.99	0.99	0.99	0.99	0.87	0.86	0.87	0.87	0.87	1.00	0.99	0.99	
T250B5	0.28	0.26	0.26	0.28	0.28	0.99	0.99	0.99	1.00	0.99	0.86	0.87	0.88	0.87	0.87	0.99	0.99	0.99	0.99

Actino-mycetes	**L05 R1**	**L05 R2**	**L05 R3**	**L05 R4**	**L05 R5**	**L05 B1**	**L05 B2**	**L0 5B3**	**L05 B4**	**L05 B5**	**T250 R1**	**T250 R2**	**T250 R3**	**T250 R4**	**T250 R5**	**T250 B1**	**T250 B2**	**T250 B3**	**T250 B4**
L05R2	1.00																		
L05R3	1.00	1.00																	
L05R4	1.00	1.00	1.00																
L05R5	1.00	1.00	1.00	1.00															
L05B1	0.29	0.30	0.30	0.28	0.30														
L05B2	0.29	0.29	0.29	0.29	0.28	0.92													
L05B3	0.29	0.29	0.28	0.28	0.29	0.92	0.91												
L05B4	0.29	0.29	0.29	0.30	0.29	0.91	0.91	0.91											
L05B5	0.31	0.29	0.30	0.29	0.29	0.91	0.90	0.89	0.90										
T250R1	0.95	0.93	0.94	0.93	0.94	0.36	0.36	0.35	0.36	0.37									
T250R2	0.95	0.94	0.95	0.94	0.96	0.35	0.36	0.35	0.36	0.36	0.98								
T250R3	0.94	0.94	0.95	0.95	0.95	0.37	0.36	0.35	0.36	0.37	0.98	0.99							
T250R4	0.95	0.95	0.95	0.94	0.95	0.37	0.35	0.37	0.37	0.36	0.99	0.99	0.99						
T250R5	0.94	0.94	0.95	0.94	0.94	0.36	0.36	0.37	0.37	0.35	0.99	0.99	0.98	0.99					
T250B1	0.87	0.87	0.86	0.88	0.87	0.82	0.82	0.82	0.83	0.82	0.68	0.69	0.70	0.68	0.69				
T250B2	0.86	0.88	0.86	0.87	0.87	0.82	0.83	0.82	0.82	0.83	0.68	0.71	0.68	0.72	0.70	1.00			
T250B3	0.88	0.87	0.87	0.86	0.88	0.82	0.83	0.82	0.81	0.83	0.70	0.70	0.69	0.71	0.71	0.99	1.00		
T250B4	0.87	0.86	0.88	0.86	0.87	0.81	0.82	0.83	0.82	0.82	0.71	0.69	0.69	0.70	0.69	1.00	1.00	1.00	
T250B5	0.86	0.87	0.86	0.88	0.86	0.82	0.81	0.82	0.83	0.82	0.69	0.69	0.69	0.71	0.71	1.00	1.00	1.00	1.00

γ-proteo bacteria	**L05 R1**	**L05 R2**	**L05 R3**	**L05 R4**	**L05 R5**	**L05 B1**	**L05 B2**	**L0 5B3**	**L05 B4**	**L05 B5**	**T250 R1**	**T250 R2**	**T250 R3**	**T250 R4**	**T250 R5**	**T250 B1**	**T250 B2**	**T250 B3**	**T250 B4**
L05R2	1.00																		
L05R3	1.00	1.00																	
L05R4	1.00	1.00	1.00																
L05R5	1.00	1.00	1.00	1.00															
L05B1	1.00	1.00	1.00	1.00	1.00														
L05B2	1.00	1.00	1.00	1.00	1.00	1.00													
L05B3	1.00	1.00	1.00	1.00	1.00	1.00	1.00												
L05B4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00											
L05B5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00										
T250R1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00									
T250R2	0.46	0.45	0.47	0.46	0.47	0.45	0.47	0.47	0.47	0.48	1.00								
T250R3	0.47	0.47	0.47	0.46	0.46	0.47	0.46	0.45	0.45	0.46	1.00	1.00							
T250R4	0.47	0.46	0.48	0.46	0.46	0.44	0.45	0.46	0.47	0.47	1.00	0.99	1.00						
T250R5	0.46	0.44	0.47	0.44	0.46	0.46	0.46	0.48	0.44	0.47	0.99	1.00	1.00	1.00					
T250B1	0.47	0.45	0.44	0.47	0.47	0.48	0.46	0.46	0.45	0.44	0.99	1.00	1.00	1.00	1.00				
T250B2	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.16	0.16	0.18	0.96	0.96	0.96	0.96	0.96	1.00			
T250B3	0.17	0.17	0.18	0.16	0.17	0.17	0.17	0.18	0.17	0.17	0.97	0.95	0.97	0.96	0.96	0.99	1.00		
T250B4	0.17	0.18	0.17	0.16	0.18	0.17	0.18	0.18	0.17	0.16	0.96	0.97	0.95	0.96	0.96	0.98	0.99	1.00	
T250B5	0.17	0.18	0.18	0.17	0.18	0.17	0.18	0.17	0.17	0.17	0.96	0.96	0.96	0.96	0.95	0.98	0.99	0.99	1.00

α-proteo bacteria	L05 R1	L05 R2	L05 R3	L05 R4	L05 R5	L05 B1	L05 B2	L0 5B3	L05 B4	L05 B5	T250 R1	T250 R2	T250 R3	T250 R4	T250 R5	T250 B1	T250 B2	T250 B3	T250 B4
L05R2	0.95																		
L05R3	0.94	0.94																	
L05R4	0.94	0.94	0.94																
L05R5	0.94	0.94	0.94	0.94															
L05B1	0.59	0.58	0.59	0.59	0.59														
L05B2	0.59	0.58	0.58	0.59	0.60	0.95													
L05B3	0.59	0.59	0.58	0.59	0.59	0.94	0.94												
L05B4	0.61	0.58	0.59	0.58	0.59	0.95	0.94	0.94											
L05B5	0.59	0.59	0.58	0.58	0.57	0.94	0.94	0.93	0.94										
T250R1	0.60	0.60	0.57	0.59	0.57	0.59	0.58	0.58	0.57	0.59									
T250R2	0.58	0.58	0.58	0.58	0.59	0.59	0.59	0.59	0.58	0.59	0.95								
T250R3	0.60	0.58	0.59	0.59	0.58	0.58	0.59	0.58	0.57	0.58	0.95	0.94							
T250R4	0.58	0.59	0.59	0.58	0.60	0.58	0.59	0.57	0.59	0.59	0.95	0.93	0.94						
T250R5	0.59	0.59	0.59	0.58	0.58	0.58	0.59	0.60	0.62	0.59	0.94	0.93	0.94	0.94					
T250B1	0.58	0.59	0.59	0.59	0.58	0.60	0.59	0.59	0.59	0.59	0.57	0.57	0.59	0.61	0.59				
T250B2	0.59	0.60	0.59	0.59	0.59	0.59	0.59	0.57	0.58	0.60	0.57	0.60	0.58	0.59	0.58	0.94			
T250B3	0.58	0.59	0.57	0.59	0.58	0.59	0.59	0.59	0.60	0.59	0.57	0.57	0.60	0.59	0.58	0.95	0.95		
T250B4	0.56	0.60	0.58	0.59	0.59	0.59	0.58	0.60	0.57	0.59	0.59	0.59	0.58	0.59	0.59	0.95	0.94	0.94	
T250B5	0.60	0.57	0.58	0.56	0.59	0.59	0.58	0.59	0.60	0.58	0.59	0.58	0.59	0.59	0.60	0.94	0.94	0.94	0.94

Figure S-1. The S_{RC} cluster analysis. Higher similarity is >0.95 and lower similarity is <0.05. Letters R and B indicate the rhizosphere and bulk soil of the Lo5 and T250 maize lines, respectively



Chapter 3

"Maize lines with different Nitrogen Use Efficiency (NUE) also differ for molecular diversity of bacterial β-glucosidase gene and glucosidase activity in their rhizosphere"

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ORIGINAL PAPER

Maize lines with different nitrogen use efficiency select bacterial communities with different β-glucosidase-encoding genes and glucosidase activity in the rhizosphere

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Abstract We studied the molecular diversity of β-glucosidase-encoding genes, microbial biomass, cellulase, *N*-acetyl-glucosaminidase, β-glucosidase, and β-galactosidase activities in the rhizosphere and bulk soil of two maize lines differing in nitrogen use efficiency (NUE). The maize lines had significant differences in diversity of β-glucosidase-encoding genes in their rhizosphere, and *Actinobacteria* and *Proteobacteria* were the dominating phyla in all samples, but representatives of *Bacteroidetes*, *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes*, and *Cyanobacteria* were also detected. Among the *Proteobacteria*, β-glucosidase genes from *α*-, *β*-, and *γ*-*Proteobacteria* were dominant in the rhizosphere of the high NUE maize line, whereas *δ*-*Proteobacteria* β-glucosidase genes were dominant in the rhizosphere of the low NUE maize line. The high NUE maize line also showed higher glucosidase activities in the rhizosphere than the low NUE maize line. We concluded that plants with high NUE select bacterial communities in the rhizosphere differing in the diversity of β-glucosidase-encoding genes which likely result in higher C-hydrolyzing enzyme activities. These effects on the diversity of β-glucosidase-encoding genes may influence the C dynamics in the agro-ecosystems.

Electronic supplementary material The online version of this article (doi:10.1007/s00374-015-1045-9) contains supplementary material, which is available to authorized users.

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Keywords β-Glucosidase-encoding genes · Molecular diversity · Glucosidase activity · Rhizosphere · Maize · Nitrogen use efficiency

Introduction

It is estimated that the majority of carbon (C) in terrestrial ecosystems is stored as soil organic matter (SOM) (Schmidt et al. 2011). Cellulose, a glucose polymer linked by β(1,4)-glucosidic bonds, is the most abundant polysaccharide in the plant residues of terrestrial environments, and its degradation due to the activity of cellulases is one of the key processes, controlling the C utilization and thus of the C biogeochemical cycle. Based on the mechanism of action, cellulases are classified as endocellulases (EC 3.2.1.4), and exocellulases (EC 3.2.1.91) also called cellobiohydrolases (CBHI and CBHII). Cellulose decomposition also involves cellobiase activity (EC 3.2.1.21), also known as β-glucosidase activity, that hydrolyzes cellulase products into glucose; this is the rate-limiting step of cellulose decomposition (Tabatabai 1982) that plays a central role in the C availability to soil microorganisms (Knight and Dick 2004). Chitin is another abundant C polymer in soil consisting of β(1,4)-glucosidic bonds between *N*-acetylglucosamine residues, being part of bacterial and fungal cell walls and insects exoskeletons, hydrolyzed by glycosyl hydrolases called chitinases (EC 3.2.1.14) produced by fungi and bacteria (Metcalfe et al. 2002).

The rhizosphere is an environmental compartment hosting fundamental processes responsible for the ecosystem functioning and crop production (Coleman et al. 1992), including cycling of C and other nutrients (Helal and Sauerbeck 1989). The rhizosphere normally hosts greater and more active microbial populations than the bulk soil, sustained by the release of root exudates that stimulate the enzyme activity in the rhizosphere

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(Baudoin et al. 2003; Renella et al. 2007). It is also known that plants select specific microbial groups in the rhizosphere and attract root symbiotic, root-associated, and root pathogenic microorganisms (Berg and Smalla 2009). In a previous study on the microbial community composition and enzymatic activities in the rhizosphere of maize (*Zea mays* L.) lines with different nitrogen use efficiency (NUE), it was reported that the maize line with higher NUE had a different microbial community and higher enzymatic activity than the low NUE maize line (Pathan et al. 2015). Carbon-hydrolyzing enzymes, such as cellulases, chitinases, and glucosidases in the rhizosphere are synthesized and released by both plants and soil microorganisms; for example, β-glucosidases, cellulases, chitinases, and other glycosyl-hydrolases are released during the colonization of plant growth-promoting rhizobacteria (Faure et al. 2001), in response to pathogen invasions (Bais et al. 2008). Owing to its very high microbial diversity, soils are a reservoir of C-hydrolyzing activities (Nannipieri et al. 2012); however, in spite of their fundamental roles in nature, the diversity of genes encoding for glycosidase enzymes is still poorly understood. In a molecular analysis of soil β-glucosidase gene diversity, Cañizares et al. (2011) reported that only few groups of microorganisms can hydrolyze cellobiose in soil and that the diversity and expression of the β-glucosidase-encoding genes varied depending on the soil plant cover and management (Cañizares et al. 2012a, b). The study of the diversity of the β-glucosidase-encoding genes in the rhizosphere of agriculturally relevant plants such as maize may be important to better understand the C dynamics in cropped soils. Moreover, to our knowledge, there are still no studies on of the diversity of β-glucosidase-encoding genes in the rhizosphere of maize plants differing for their N use efficiency (NUE). We therefore studied the diversity of the β-glucosidase genes and cellulase, chitinases, β-glucosidase, and β-galactosidase activities in the rhizosphere and bulk soil of two maize lines differing for their NUE. The starting hypotheses of this research were as follows: (i) plants differing for NUE selected microbial communities in the rhizosphere with different bacterial β-glucosidase-encoding genes and (ii) microbial communities differing for bacterial β-glucosidase-encoding genes could result in differences in β-glucosidase activity and other enzyme activities involved in organic C mineralization such as β-galactosidase, *N*-glucosaminidase, and cellulase. We tested our hypotheses by studying the rhizosphere and bulk soil of maize plants with different NUE, grown in rhizoboxes allowing precise sampling of the rhizosphere and bulk soil.

Materials and methods

Soil properties, maize plants, and rhizobox setup

A sandy clay loam soil classified as a Eutric Cambisol (World Reference Base for Soil Resources 2006), under conventional maize crop regime, located at Cesa (Tuscany, Central Italy), was sampled from the Ap horizon (0–25 cm). The soil was sieved at field moisture (<2 mm), after removing visible plant material. Soil had a pH value (in H_2O) of 7.1, contained 32.1 % sand, 42.2 % silt, 25.7 % clay, 10.8 g kg^{-1} total organic C (TOC), 1.12 g kg^{-1} total N, and 6.45 g kg^{-1} total P. After sieving, 600 g of soil was placed in the soil compartment of the rhizoboxes. The rhizoboxes consisted of two bulk soil compartments separated by the plant compartment, enclosed by 0.22-μm mesh nylon tissue. Full details on the used rhizoboxes and maize growth conditions have been already reported (Pathan et al. 2015). The Lo5 and T250 maize lines, having high and low NUEs, respectively, were grown for 21 and 28 days, respectively, a suitable growth period to allow the full colonization of the plant compartment by plant roots and prevent nutrient starvation, as resulted from previous experiments (Pathan et al. 2015). Plants were regularly watered with distilled sterile H_2O, and no fertilizers were applied during the plant growth. All rhizoboxes were prepared in five replicates for each maize line. The used rhizoboxes allowed precise sampling of rhizosphere due to the presence of fixed sampling grooves at precise increment distances from the surface of the plant compartment. Rhizosphere (R) and bulk soil (B) samples of the Lo5 and T250 maize lines were named as Lo5R, Lo5B, T250R, and T250B, respectively. Rhizosphere and bulk samples were kept separate after sampling and immediately analyzed for the enzyme activities or stored at −80 °C before ATP determination or DNA extraction.

Soil microbial biomass and enzyme activities

Microbial biomass was determined by measuring the soil ATP content according to Ciardi and Nannipieri (1990). Cellulase activity was estimated by using the 4-nitrophenyl-β-D-cellobioside (SIGMA) as substrate, in modified universal buffer (MUB) at pH 5.0 for 2 h using 2-g soil (dry weight (d.w.) equivalent). The *N*-acetyl-β-D-glucosaminidase (NAGase) activity (EC 3.2.1.30) was estimated by using 4-nitrophenyl-β-D-glucosaminidine (SIGMA) as substrate, in 0.1 acetate buffer at pH 5.2 for 1 h, using 1 g d.w. soil. Both cellulase and NAGase activities were determined at 50 °C that, from preliminary experiments, was found to be the optimal temperature for these two enzyme activities. The β-glucosidase and β-galactosidase activities were measured using 4-nitrophenyl-β-D-glucopyranoside and 4-nitrophenyl-β-D-galactopyranoside as substrates, respectively, according to Tabatabai (1982). Concentrations of p-nitrophenol (p-NP) produced by the enzyme activity assays were calculated from a p-NP calibration curve after subtraction of the absorbance of the controls at 400-nm wavelength.

DNA extraction and PCR-DGGE analyses

Total soil DNA was extracted from all the five independent replicate rhizoboxes for each maize line by the sequential extraction method described by Ascher et al. (2009) and quantified with a Qubit® 2.0 flurometer (Invitrogen, USA). Molecular weight and fragment length of DNA were checked on 1.5 % agarose gel. The primers GC-βgluF2 (TTCYTBGGYRTCAACTACTA) and βgluR4 (CCGTTYTCGGTBAYSWAGA) were used to amplify the β-glucosidase-encoding genes by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), according to Cañizares et al. (2011), and amplicon size was 219 bp. Forty nanograms of DNA template was amplified with 5 U μl^{-1} Dream Taq DNA Polymerase (Thermo Scientific), 10 μM of each primer, 10 mM of each dNTPs, 10× (plus $MgCl_2$ 20 mM) Dream Taq reaction buffer (Thermo Scientific), and 500 $\mu g\ ml^{-1}$ BSA, in a final reaction volume of 50 μl. A TProfessional Thermocycler (Biometra) was used for PCR amplification. The PCR program employed was the same as that by Cañizares et al. (2011), with the only modification of lower number of cycles: 5-min denaturation at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 53 °C, 1 min at 72 °C, followed by final extension step at 72 °C for 10 min. DGGE analysis was performed with the Ingeny PhorU system (Ingeny, International BV, Netherlands). Briefly, 100 ng of PCR products was loaded into 8 % (*w*/*v*) polyacrylamide gel, with a urea denaturing gradient from 40 to 65 %. Electrophoresis was performed in 1× TAE buffer at 58 °C for 6 h at 150 V. The gel was stained with SYBR Green for 40–45 min and analyzed by Gel DOC™ XR+ system (Bio-Rad, Hercules, CA, USA).

Diversity of the β-glucosidase-encoding genes

Degenerate primers βgluF2/βgluR4 were used to amplify the conserved motif of soil bacterial β-glucosidase-encoding genes. The PCR program employed was the same as that by Cañizares et al. (2011), and amplicon size was 180 bp. The PCR products were separated by gel electrophoresis, gel purified using Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel, Germany), and quantified by a Qubit® 2.0 fluorometer (Invitrogen, USA). Purified DNA from five replicates of each treatment was pooled together, and 1 μg of DNA was used for library preparation and sequencing, according to the Illumina sample preparation guide (http://supportres.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Paired-end sequencing (2× 150 bp) was carried out using Illumina Miseq sequencer (BGI Tech Solution, Hong Kong).

Paired reads were assembled and filtered out using biopieces (www.biopieces.org). Forward and reverse primers were trimmed, and the quality of reads was checked with biopieces (www.biopieces.org). Mismatches between the overlapping fragments of forward and reveres reads were corrected according to the base call with the higher sequence-assigned quality score (>35). Denoising was performed with 97 % similarity clustering with heuristic clustering algorithm UCLUST, implemented in USEARCH v7.01.1001, and all reads were de-replicated using biopieces (www.biopieces.org). Chimeric sequences were filtered and removed using biopieces. The operational taxonomic units (OTUs) with less than two reads in common were excluded from the analysis. For identification, representative sequences were blasted against CAZy database (http://mothra.ornl.gov/cgi-bin/cat/cat_v2.cgi?tab=ORTHOLOGS, www.cazy.org), and accession numbers from blast outputs were retrieved in Batch Entrez (www.ncbi.nlm.nih.gov/sites/batchentez) for taxonomic assignment. CAZy is a widely used dataset which contains information on enzymes involved in breakdown of carbohydrates, modification, and synthesis of glycosidic bonds. The CAZy classification covered many enzymes classes such as glycoside hydrolases (GH), carbohydrate esterases (CE), polysaccharide lyases (PL), and glycosyltransferases (GT). The CAZy is updated daily and provides the richest set of manually curated information about all groups of CAZymes, including their names, gene accessions, EC numbers, and 3D structure and taxonomy.

Rarefaction curves based on identified OTUs were plotted, and the diversity indices of Shannon and Weaver (1948) and Simpson (1949) were calculated using Paleontological Statistic (PAST, version 3.X) software (Hammer et al. 2001).

Data analysis

The analysis of the ATP content and enzyme activities were conducted by ANOVA followed by the Fisher-protected LSD test (FPLSD), using the Statview® software (SAS Institute Inc., USA). The DGGE banding patterns were compared using the Quantity-One® software (Bio-Rad Laboratories, USA), with the lanes normalized considering the total signal after background subtraction. Band positions were converted to Rf values between 0 and 1, and profile similarity was calculated by determining Dice's similarity coefficient (Sørensen 1948) for the total number of lane patterns. Calculated similarity coefficients were then used to construct a dendrogram using the unweighted pair group method with arithmetical averages (UPGMA).

Illumina sequencing data analysis

Species richness was calculated by considering at least 2000 sequences, and sequences were considered similar by using the commonly used cutoff value of 97 % for comparative analysis of sequences (Konstantinidis et al. 2006). ANOVA

followed by Tukey post hoc test was also performed on diversity indices to evaluate statistical differences among all four different conditions. Relative abundance of taxonomic phylum (i.e., phyla and sub-classes) was also estimated by dividing the number of sequences belonging to each phylogenetic group by the total number of sequences in given samples. To visualize the diversity of β-glucosidase gene sequences, we performed dual hierarchical clustering and constructed a heatmap with the heatmap.2 function in the gplot package of the R Statistics Environment (R Development Core Team 2008). Heatmap showed OTUs which are only identified at species level. Data were hierarchically clustered using Euclidean distance matrix. Principal coordination analysis (PCoA) based on Raup and Crick distance matrix (S_{RC}) (Raup and Crick 1979) was performed to evaluate similarities or dissimilarities within and between soils collected under different plant varieties. The S_{RC} value is the probability that the randomized similarity would be greater or equal to the observed similarity. The S_{RC} values were calculated using PAST software (Hammer et al. 2001), and S_{RC} values above 0.95 or below 0.05 indicated significant similarity or dissimilarity, respectively. The PCoA was calculated using XLSTAT (ADDINSOFT SARL) software. PCoA allows to analyze dissimilarities between samples according to a phylogenetic measure (Ramette 2007). The relationship between β-glucosidase-encoding gene diversity and soil properties was determined by canonical correspondence analysis (CCA) in CANOCO 4.5 (Ter Braak and Smilauer 2002). The Monte Carlo permutation test (499 random permutations) was performed to analyze significant effects of environmental variables on the observed β-glucosidase-encoding gene diversity. Soil chemical and biochemical properties were represented as vectors with length and slope as substantial constraints. Contents of soil TOC, NH_4^+-N, NO_3^--N, ATP, and available P were taken from the previous study on the same soil samples from which the DNA was extracted (Pathan et al. 2015).

Results

Soil ATP content and enzyme activities

The β-glucosidase, β-galactosidase, cellulase, and NAGase activities were significantly higher in the rhizosphere than in bulk soil of both the Lo 5 and T 250 maize lines, except for the β-galactosidase activity in the T 250 maize line which was not significantly different between rhizosphere and bulk soil (Table 1). The rhizosphere of the Lo5 maize line showed the highest values for the measured four C-hydrolyzing enzyme activities except for the cellulase activity, which was not significantly different between the rhizosphere of the two maize lines (Table 1). The bulk soils of the two maize lines showed similar C-hydrolyzing enzyme activities except for the β-glucosidase activity that was significantly higher for the Lo 5 than the T 250 maize line (Table 1). The ATP content was significantly higher in the rhizosphere than in bulk soil of both the Lo 5 and T 250 maize lines, with the rhizosphere of the Lo 5 maize line showing significantly higher values than the T 250 maize (Table 1).

Diversity of the β-glucosidase-encoding genes

The DGGE analysis revealed complex banding patterns in the rhizosphere and bulk soil of both maize lines, and the UPGMA dendrogram showed that the rhizosphere and bulk soils of the two maize lines clustered separately, with a high similarity between the β-glucosidase-encoding genes in the bulk soil of the two plants (Fig. 1). Probably, the observed biological variability was low also due to the low sensitivity of the PCR-DGGE technique that we used.

The detected OTU numbers were 2944 for Lo 5R, 5607 for Lo 5B, 4884 for T 250R, and 5472 for T 250B. Rarefaction curves of rhizosphere and bulk soil of the Lo 5 and T 250 maize lines showed that as the number of sequences increased, the number of OTUs approached a plateau, indicating a reliable estimation of the diversity of β-glucosidase-encoding genes in the rhizosphere and bulk soil of both maize lines (Supplementary Fig. 1). The number of OTUs for similar numbers of sequences was always higher in bulk than the rhizosphere soils of both maize lines, and the Lo 5 presented a lower number of OTUs than the rhizosphere of T 250 maize line. Calculation of the values of the Shannon-Weaver and Simpson indices of diversity showed that the β-glucosidase-encoding gene diversity was lower in the rhizosphere than in bulk soil for both maize lines (Table 2).

Taxonomic affiliation was only performed on OTU data, which were identified at genus and species level, and using species and genus information, classification was done at phylum and class level (Fig. 2a, b). The dominant phyla were *Actinobacteria* (76 % in Lo 5R, 68 % in Lo 5B, 64 % in T 250R, 56.0 % in T 250B), followed by *Proteobacteria* (23 % in Lo 5R, 31 % in Lo 5B, 34 % in T 250R, 32 % in T 250B), and sequences belonging to *Bacteroidetes*, *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes*, and *Cyanobacteria* phylogenetic groups were also detected. Sequences from *Cyanobacteria* were only observed in T 250B (8 %), and sequences from *Chloroflexi* were higher in T 250B (3 %) than the other soils. Among the *Proteobacteria*, sequences clustered into α-, β-, γ-, and δ-sub-classes (Fig. 2b). Sequences from α-, β-, and γ-*Proteobacteria* were dominant in the Lo5 maize line, whereas δ-*Proteobacteria* were the most abundant in the T250 maize line. At genus level, *Rubrobacter* (*Actinobacteria*) was the most abundant in the rhizosphere of both maize lines.

The heatmap showed the distribution pattern of β-glucosidase-encoding gene diversity at species level in

Table 1 Enzyme activities in the rhizosphere and bulk soil of the Lo5 and T 250 maize lines after the growth period

Soil	β-Glucosidase (mg p-NP kg^{-1} h^{-1})	β-Galactosidase (mg p-NP kg^{-1} h^{-1})	Cellulase (mg p-NP kg^{-1} h^{-1})	NAGase (mg p-NP kg^{-1} h^{-1})	ATP (ng kg^{-1})
Lo5 R	365.8^{a}	158.5^{a}	150.8^{a}	41.9^{a}	348.3^{a}
Lo5 B	169.3^{c}	113.8^{b}	83.8^{b}	31.4^{c}	259.9^{c}
T 250 R	193.4^{b}	112.5^{b}	130.6^{a}	35.7^{b}	286.9^{b}
T 250 B	140.2^{d}	102.3^{b}	88.3^{b}	30.6^{c}	263.9^{c}

Different letters indicate significant differences among mean values in columns

NAGase N-acetyl-glucosaminidase activity

individual soil samples (Fig. 3). Rhizosphere soils from both maize lines clustered together whereas bulk soil from both maize lines clustered independently from the rhizosphere soils. The rhizosphere of the T 250 maize line was characterized by highly abundant OTUs identified as sequences of *Anoxybacillus flavithermus* (ACJ34717), *Pelagibacterium halotolerans* (AEQ50474), *Variovorax* sp. (AFC17958), *Salinispora arenicola* (ABV97405), *Rhodobacter capsulatus* (ADE85514), and *Streptomyces scabies* (CBG72797), whereas the rhizosphere of the Lo 5 maize line was characterized by high abundance of OTUs from *Arthrobacter aurescens* (ABM06312).

The OTUs identified with reference sequences as *Haliangium ochraceum* (ACY18557), *Verrucosispora maris* (AFC17958), *Microbacterium* sp. (AFC17955), *Pseudomonas* sp. (AFC17959), *Streptomyces* sp. (AFO59750), *Desulfobulbus propionicus* (ADW18818), *Amycolatopsis mediterranei* (ADJ41910, ADJ44240), *Streptomyces hygroscopicus* (AEY89292, AGF63450), *Cellulomonas biazotea* (AEM45802), *Streptomyces coelicolor* (NP_626770), *Sorangium cellulosum* (CAN944460, CAN97832), *Saccharophagus degradans* (ABD80656), *Mycobacterium smegmatis* (AFP41454), *Halobacillus halophilus* (CCG46555), and *Gloeocapsa* sp. (AFZ29126) were only detected in the bulk soil of the T250 maize line, whereas the OTUs identified with sequences from *Streptomyces griseus* (BAG17200, BAG18260), *Streptomyces hygroscopicus* (AEY89292, AGF63450), *Amycolatopsis mediterranei* (AFO73620, AFO75953), and *Anaerocellum thermophilum* (BAJ63148) were the most abundant in the bulk soil of the Lo5 maize line.

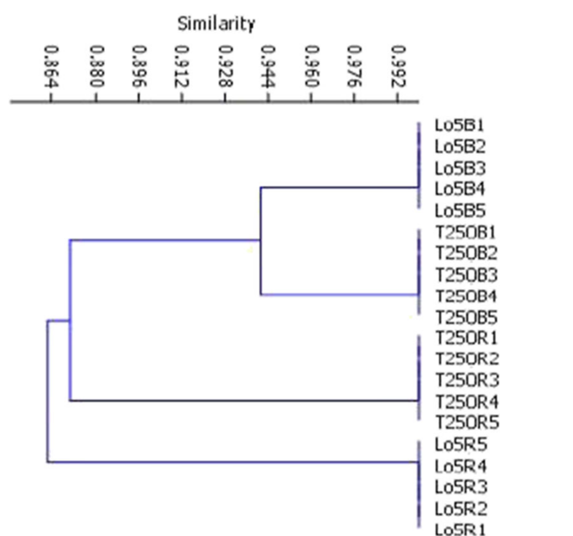

Fig. 1 Dice similarity coefficient-based UPGMA dendrogram for the bacterial β-glucosidase gene DGGE profile. The letters *R* and *B* indicate rhizosphere and bulk soil, respectively

The PCoA showed that β-glucosidase-encoding gene diversity of rhizosphere soils from both maize lines and bulk soil from Lo 5 maize line clustered together, whereas the β-glucosidase-encoding gene diversity of bulk soil from T 250 maize line clustered separately (Supplementary Fig. 2). This result was confirmed by the S_{RC} values showing a significant similarity (S_{RC}>0.95) between β-glucosidase-encoding genes of rhizosphere of the Lo 5 and T 250 maize lines and also with the Lo 5 bulk soil (Table 3). A significant dissimilarity (S_{RC}< 0.95) was observed between the diversity of β-glucosidase-encoding genes of bulk soils of the two maize lines (Table 3).

Relationship between β-glucosidase-encoding gene diversity, soil properties, and enzyme activities

A strong relationship between the diversity of β-glucosidase-encoding genes and soil chemical and biochemical properties was found by CCA analysis, as the first two axes accounted for 75.2 and 13.8 % of the total variance, indicating soil properties (Fig. 4). Moreover, in the rhizosphere of both Lo 5 and T 250 maize lines, a significant correlation between β-

Table 2 Shannon and Simpson indices of molecular diversity of bacterial β-glucosidase-encoding genes in the rhizosphere and bulk soil of the Lo5 and T 250 maize lines after the growth period

Soil	Indices	
	Shannon-Weaver	Simpson
Lo5 R	1.39^{a}	0.50^{a}
Lo5 B	1.70^{b}	0.61^{b}
T 250 R	1.76^{b}	0.62^{b}
T 250 B	1.97^{c}	0.69^{c}

Different letters indicate significant differences among mean values in columns

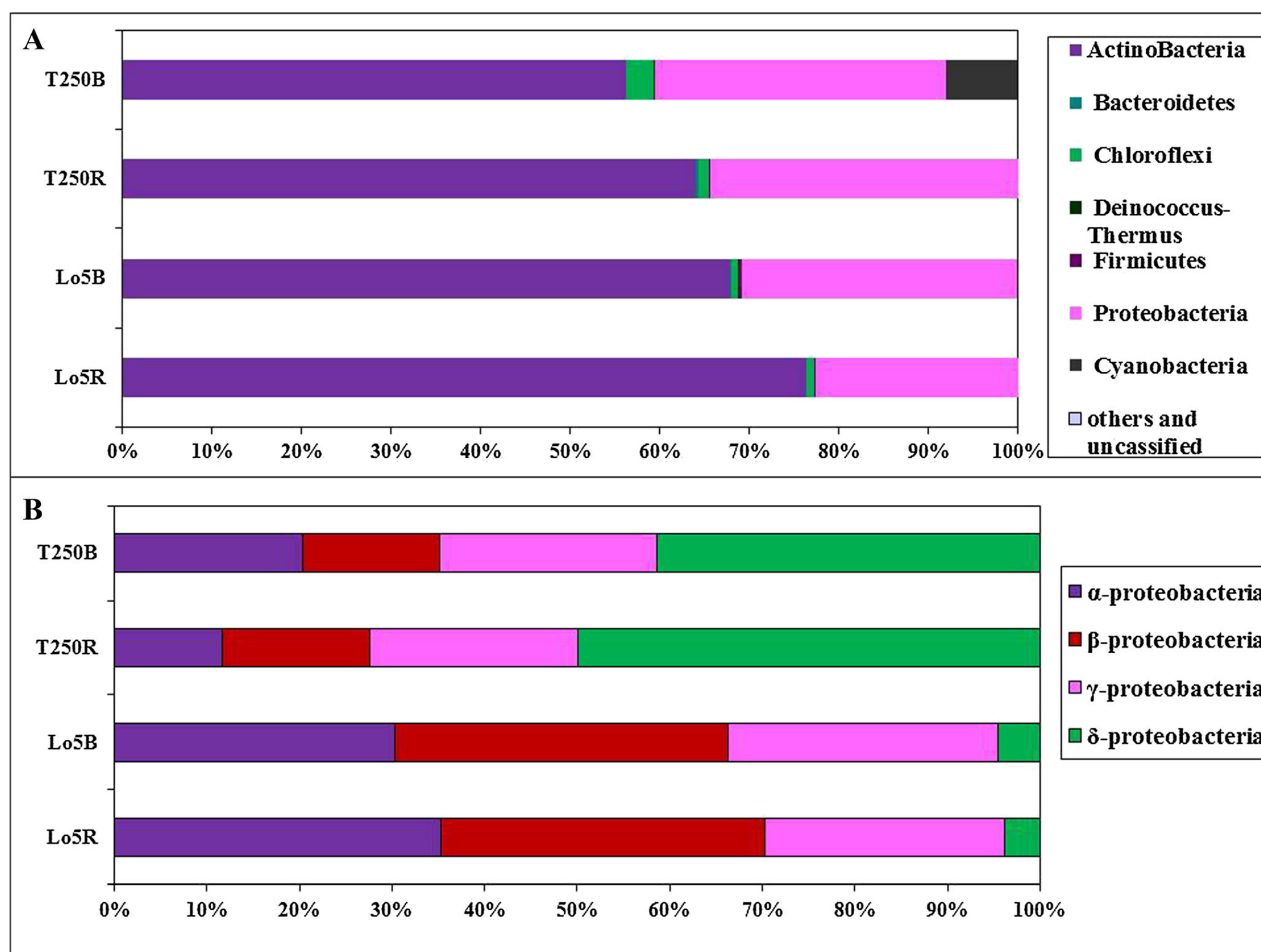

Fig. 2 Taxonomic classification of molecular diversity of bacterial β-glucosidase-encoding gene sequences at the phylum level (**a**) and at the class level for *Proteobacteria* (**b**) in rhizosphere and bulk soil of Lo5 and T250 inbred maize lines. The letters *R* and *B* indicate rhizosphere and bulk soil, respectively

glucosidase-encoding gene diversity and soil ATP content and enzyme activities and concentrations of TOC, available P, and NO_3^--N was also observed (Fig. 4). Differently, the diversity of β-glucosidase-encoding genes of bulk soils of both maize lines was not related to soil chemical and biochemical properties, whereas the NH_4^+-N concentration was not related to the diversity of the β-glucosidase-encoding genes in any case (Fig. 4).

Discussion

The high NUE Lo 5 maize line had higher enzyme activity and microbial biomass in the rhizosphere than the T 250 maize line, confirming previous results (Pathan et al. 2015). This is interesting because the higher cellulase-hydrolyzing activities could indicate a faster SOM decomposition in the rhizosphere of the high NUE Lo 5 than in low NUE T 250 maize line (Bandick and Dick 1999; Bowen and Rovira 1991). The higher C hydrolytic activities in the rhizosphere could contribute to nutrient acquisition by plants by accelerating the SOM turnover (Nannipieri et al. 2012). The increase in β-glucosidase, cellulase, and β-galactosidase activities could also be due to the stimulation of microbial enzyme synthesis upon the release of glucosides and galactosides in the rhizosphere (Lugtenberg and Bloemberg 2004), whereas the higher *N*-acetyl-glucosaminidase activity could depend on the release of chitinases for controlling fungal pathogens, attracting plant-beneficial microorganisms, and solubilizing nutrients in the rhizosphere (Bais et al. 2008; Badalucco and Nannipieri 2007). However, these hypotheses should to be verified by future research on the root exudate profiles of the studied maize lines.

Our results also showed that maize lines with different NUE also differed in the diversity of β-glucosidase genes in the rhizosphere, with *Actinobacteria* and *α*-, *β*-, and *γ*-*Proteobacteria* being most abundant phylogenetic groups

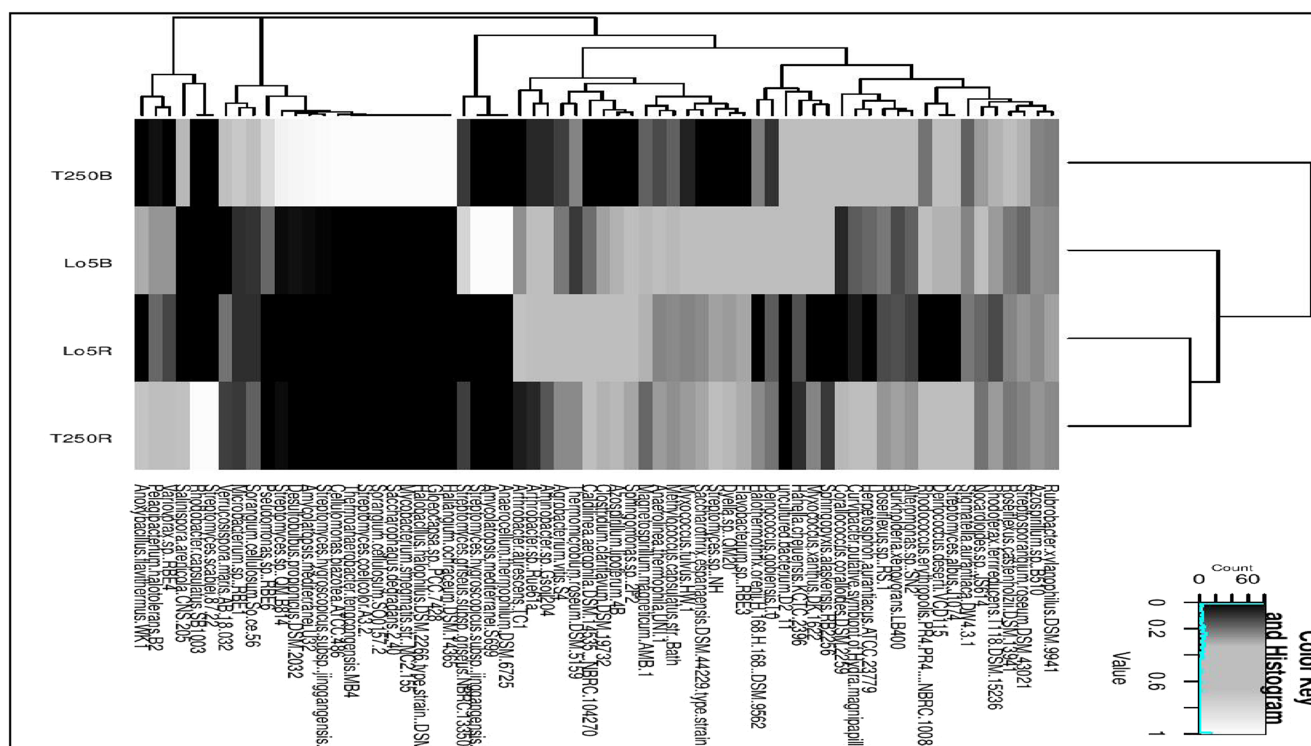

Fig. 3 Heatmap of bacterial β-glucosidase-encoding gene sequences in rhizosphere and bulk soil of Lo5 and T250 maize varieties. The letters *R* and *B* indicate rhizosphere and bulk, respectively, *light grey* to *black color* indicating high to low abundance of species in different soils

carrying β-glucosidase genes. These results agreed with previous reports (Chelius and Triplett 2001; Cañizares et al. 2011; Chauhan et al. 2011; Li et al. 2014), and *δ-Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes*, and *Cyanobacteria* were detected at low frequency, as reported by Chelius and Triplett (2001) (Fig. 2b). By considering the previous results by Pathan et al. (2015) who showed that the two studied maize lines host different microbial communities in their rhizosphere, these results indicated that plants can influence the diversity of key functional genes in the rhizosphere.

Some of the identified prokaryotes have been previously detected in soils under conventional maize cultivation and also known as maize endophytes (Thanh and Diep 2014). For example, *Variovorax* sp., *Arthrobacter aurescens*, *P. halotolerans*, and *Streptomyces coelicolor* have been detected as maize root associates capable of pesticide and herbicide degradation (Barriuso et al. 2010; Xu et al. 2011), and *Microbacterium testaceum* is a maize endophyte (Zinniel et al. 2002). Detection of these species reflects the past use of the tested soil for conventional maize cultivation. Other detected OTUs such as *Rhodobacter capsulatus* and *Anoxybacillus flavithermus* have been shown to have plant growth-promoting activity and to stimulate root N uptake of other cereal crop plants such as barley (Cakmacki et al. 1999) and rice (Hongrittipun et al. 2014). *Sorangium cellulosum* has been found in the rhizosphere of woody plants (Uroz et al. 2010). Another important finding is that the most abundant strains carrying the β-glucosidase-encoding genes were sugar-degrading bacteria (e.g., *Amycolatopsis mediterranei*, *Streptomyces hygroscopicus*, *Anoxybacillus flavithermus*), which have been previously isolated from both rhizosphere and bulk soil (Pikuta et al. 2000; Gonzalez-Franco et al. 2003; Duangmal et al. 2011). Moreover, these prokaryotes are also neutrophilic/alkaliphilic species, therefore well adapted to the sub-alkaline studied soil. Overall, our results not only confirmed that different plant genotypes can change the diversity of the rhizosphere microorganisms (Berg and Smalla 2009), but also clearly indicate that they also significantly influence the functional diversity in the rhizosphere.

Our results also showed that the two maize lines had lower OTUs in the rhizosphere than in bulk soil, indicating a strong rhizosphere selection of the soil microbial communities.

Table 3 Raup and Crick probability similarity index and values (S_{RC}) for β-glucosidase-encoding genes in the rhizosphere and bulk soil of the Lo5 and T 250 maize lines after the growth period

Soil	Lo5 R	Lo5 B	T 250 R
Lo5 B	*0.97*	–	–
T 250 R	*1.00*	0.93	–
T 250 B	0.19	*0.002*	0.22

Values in italics higher >0.95 and <0.05 indicate significant similarity and significant dissimilarity, respectively

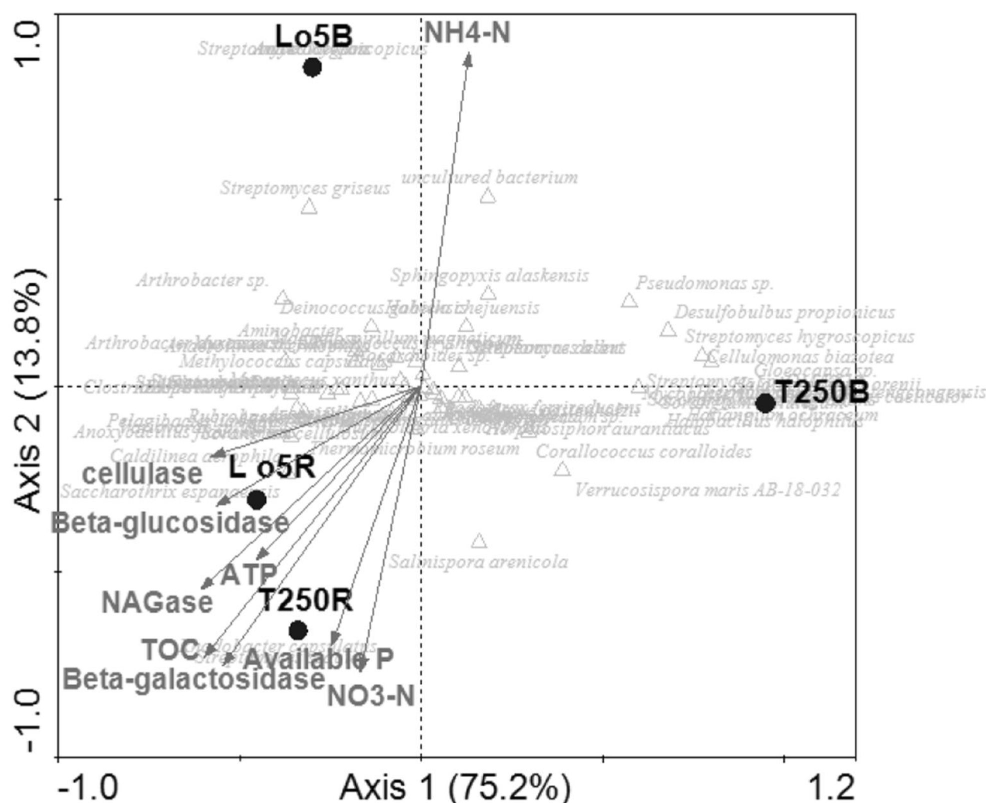

Fig. 4 Canonical correspondence analysis (CCA) of β-glucosidase gene sequences in rhizosphere and bulk soil of Lo5 and T250 maize varieties. *R* and *B* indicate rhizosphere and bulk respectively. Vectors indicate the different weight and orientation of environmental variable. *NAGase N*-acetyl-glucosaminidase, *Beta-glucosidase* β-glucosidase, *Beta-galactosidase* β-galactosidase, *TOC* total organic C

These results confirm that the rhizosphere is a selective environment (Berg and Smalla 2009), likely due to the release of root exudates (Landi et al. 2006). The lower OTU numbers in the rhizosphere of the Lo 5 than in the T 250 maize line suggest a stronger microbial selection by the maize line with the higher NUE, paralleling those of the higher C-hydrolyzing enzyme activities, indicating that the selected rhizosphere bacterial communities of the high NUE Lo 5 maize line have a greater glycolytic potential than those of the low NUE T 250 maize line. To our knowledge, these results are the first showing that the rhizospheres of plants differing in NUE are also characterized by differences in the diversity of key functional genes such as those encoding for the β-glucosidase activity and that this selection is related to higher glycolytic activity in the rhizosphere, and further investigation with a transcriptomic approach is needed to better understand the relation between microbial community composition and enzyme activity in rhizosphere and maize NUE.

It should be also mentioned that the measured cellulase, NAGase, and β-glucosidase activities are interrelated, because the first two enzyme activities supply substrates for the third one by releasing cellobiose from cellulose and hydrolyzing β-l,4-glycosidic bonds of chitin (Sasaki et al. 2002), and therefore, high cellulase and NAGase activities could, in turn, influence the diversity of β-glucosidase-encoding genes. However, because the rate of expression of β-glucosidase genes from different microorganisms can be independent on their relative abundance in soil (Baldrian et al. 2012), analysis of gene expression at transcriptomic and proteomic levels is needed to better understand the link between the diversity of β-glucosidase-encoding genes and β-glucosidase in the rhizosphere of plants differing for NUE. Moreover, because the CCA analysis also showed that soil chemical properties were also significantly related to the diversity of the β-glucosidase-encoding genes of rhizosphere soil (Fig. 4), the analysis of β-glucosidase gene expression will also contribute to elucidate the relative importance of main factors such as root exudate profiles, microbial community composition, and soil properties controlling the β-glucosidase activity in the rhizosphere.

In conclusion, our results confirmed that plants can select microbial communities in the rhizosphere and can also select key functional genes such as those encoding β-glucosidase activity. It was also shown that maize lines with higher NUE induce a stronger selection of bacterial groups carrying β-glucosidase-encoding genes and that this selection was related to higher C-hydrolyzing enzyme activities in the rhizosphere. Future research is needed to characterize the root exudate profiles of the studied maize lines to better explain the bacterial selection mechanisms in the rhizosphere and also to determine the expression of β-glucosidase-, cellulase-, and chitinase-encoding genes at both mRNA and protein levels.

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References

Ascher J, Ceccherini MT, Pantani OL, Agnelli A, Borgogni F, Guerri G, Nannipieri P, Pietramellara G (2009) Sequential extraction and fingerprinting of soil metagenome. Appl Soil Ecol 42:176–181

Badalucco L, Nannipieri P (2007) Nutrient transformations in the rhizosphere. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere: biochemistry and organic substances at the soil–plant interface, 2nd edn. CRC Press, Boca Raton, pp 111–133

Bais HP, Broeckling CD, Vivanco JM (2008) Root exudates modulate plant-microbe interaction in the rhizosphere. In: Karlovsky P (ed) Secondary metabolites in soil ecology, soil biology 14. Springer, Berlin, pp 241–252

Baldrian P, Kolarık, Stursova M, Kopecky J, Valaskova V, Vetrovsky T, Zifcakova L, Snajdr J, Rıdl J, Vlcek C, Vorıskova J (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J 6:248–258

Bandick AK, Dick R (1999) Field management effects on soil enzyme activities. Soil Biol Biochem 31:1471–1479

Barriuso J, Marín S, Mellado RP (2010) Effect of the herbicide glyphosate on glyphosate-tolerant maize rhizobacterial communities: a comparison with pre-emergency applied herbicide consisting of a combination of acetochlor and terbuthylazine. Environ Microbiol 12:1021–1030

Baudoin E, Benizri E, Guckert A (2003) Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biol Biochem 35:1183–1192

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Bowen GD, Rovira A (1991) The rhizosphere. In: Walsel Y, Eshel A, Kafkafi U (eds) Plants root, the hidden half. Marcel Dekker, New York, pp 641–669

Cakmacki R, Kantar F, Algur OF (1999) Sugar beet and barley yields in relation to *Bacillus polymyxa* and *Bacillus megaterium* var. *phosphaticum* inoculation. J Plant Nutr Soil Sci 162:437–442

Cañizares R, Benitez E, Ogunseitan OA (2011) Molecular analyses of beta-glucosidase diversity and function in soil. Eur J Soil Biol 47:1–8

Cañizares R, Moreno B, Benitez E (2012a) Biochemical characterization with detection and expression of bacterial β-glucosidase encoding genes of a Mediterranean soil under different long term management practices. Biol Fertil Soils 48:651–663

Cañizares R, Moreno B, Benitez E (2012b) Bacterial β-glucosidase function and metabolic activity depend on soil management in semiarid rainfed agriculture. Ecol Evol 2:727–731

Chauhan PS, Chaudhry V, Mishra S, Nautiyal CS (2011) Uncultured bacterial diversity in tropical maize (*Zea mays* L.) rhizosphere. J Basic Microbiol 51:15–32

Chelius MK, Triplett EW (2001) The diversity of archaea and bacteria in association with the roots of *Zea mays* L. Microb Ecol 41:252–263

Ciardi C, Nannipieri P (1990) A comparison of methods for measuring ATP in soil. Soil Biol Biochem 22:725–727

Coleman DC, Odum EP, Crossley DA (1992) Soil biology, soil ecology and global change. Biol Fertil Soils 14:104–111

R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org

Duangmal K, Mingma R, Pathom-aree W, Thamchaipenet A, Inahashi Y, Matsumoto A, Takahashi Y (2011) Amycolatopsis samaneae sp. nov., isolated from roots of *Samanea saman* (Jacq.) Merr. Int J Syst Evol Microbiol 61:951–955

Faure D, Henrissat B, Ptacek D, Bekri MA, Vanderleyden J (2001) The celA gene, encoding a glycosyl hydrolase family 3 β-glucosidase in *Azospirillum irakense*, is required for optimal growth on cellobiosides. Appl Environ Microbiol 67:2380–2383

Gonzalez-Franco AC, Deobald LA, Spivak A, Crawford DL (2003) Actinobacterial chitinase-like enzymes: profiles of rhizosphere versus non-rhizosphere isolates. Can J Microbiol 49:683–698

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistic software package for education and data analysis. Palaeontol Electron 4:1–9

Helal HM, Sauerbeck D (1989) Carbon turnover in the rhizosphere. Z Pflanzenernaehr Bodenkd 152:211–216

Hongrittipun P, Youpensuk S, Rerkasem B (2014) Screening of nitrogen fixing endophytic bacteria in *Oryza sativa* L. J Agric Sci 6:66–74

Knight TR, Dick RP (2004) Differentiating microbial and stabilized β-glucosidase activity relative to soil quality. Soil Biol Biochem 36: 2089–2096

Konstantinidis KT, Ramette A, Tiedje JM (2006) Toward a more robust assessment of intraspecies diversity, using fewer genetic markers. Appl Environ Microbiol 72:7286–7293

Landi L, Valori F, Ascher J, Renella G, Falchini L, Nannipieri P (2006) Root exudate effects on the bacterial communities CO_2 evolution nitrogen transformations and ATP content of rhizosphere and bulk soils. Soil Biol Biochem 38:509–516

Li X, Rui J, Xiong J, Li J, He Z, Zhou J, Yannarell AC, Mackie RI (2014) Functional potential of soil microbial communities in the maize rhizosphere. PLoS ONE 9, e112609

Lugtenberg BJJ, Bloemberg GV (2004) Life in the rhizosphere. In: Ramos JL (ed) Pseudomonas. Kluwer Academic/Plenum Publishers, New York, pp 403–430

Metcalfe AC, Krsek M, Gooday GW, Prosser JI, Wellington EMH (2002) Molecular analysis of a bacterial chitinolytic community in an upland pasture. Appl Environ Microbiol 68:5042–5050

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Fornasier F, Moscatelli MC, Marinari S (2012) Soil enzymology: classical and molecular approaches. Biol Fertil Soils 48:743–762

Pathan SI, Ceccherini MT, Pietramellara G, Puschenreiter M, Giagnoni L, Arenella M, Varanini Z, Nannpieri P, Renella G (2015) Enzyme activity and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency. Plant Soil 387:413–424

Pikuta E, Lysenko A, Chuvilskaya N, Mendrock U, Hippe H, Suzina N, Nikitin D, Osipov G, Laurinavichius K (2000) *Anoxybacillus pushchinensis* gen. nov., sp. nov., a novel anaerobic, alkaliphilic, moderately thermophilic bacterium from manure, and description of *Anoxybacillus flavithermus* comb. nov. Int J Syst Evol Microbiol 50:2109–2117

Ramette A (2007) Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62:142–160

Raup DM, Crick RE (1979) Measurement of faunal similarity in paleontology. J Paleontol 53:1213–1227

Renella G, Landi L, Valori F, Nannipieri P (2007) Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clay and a sandy soil. Appl Soil Ecol 36:124–129

Sasaki C, Yokoyama A, Itoh Y, Hashimoto M, Watanabe T, Fukamizo T (2002) Comparative study of the reaction mechanism of family 18 chitinases from plants and microbes. J Biochem 13:557–564

Schmidt MI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DA,

Nannipieri P, Rasse DP, Weiner S, Trumbore SE (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56

Shannon CE, Weaver W (1948) A mathematical theory of communication. Bell Syst Tech J 27379–27423

Simpson EH (1949) Measurement of diversity. Nature 163:688

Sørensen T (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. K Dan Vidensk Selsk Biol Skr 5:1–34

Tabatabai MA, Soil enzymes (1982) In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, part 2, chemical and microbiological properties, 2nd edn. American Society of Agronomy/Soil Science Society of America, Madison, pp 903–947

Ter Braak CJF, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power Ithaca. Available at http://www.canoco.com

Thanh DTN, Diep CN (2014) Isolation, characterization and identification of endophytic bacteria in maize (*Zea mays* L.) cultivated on Acrisols of the Southeast of Vietnam. Am J Life Sci 2:224–233

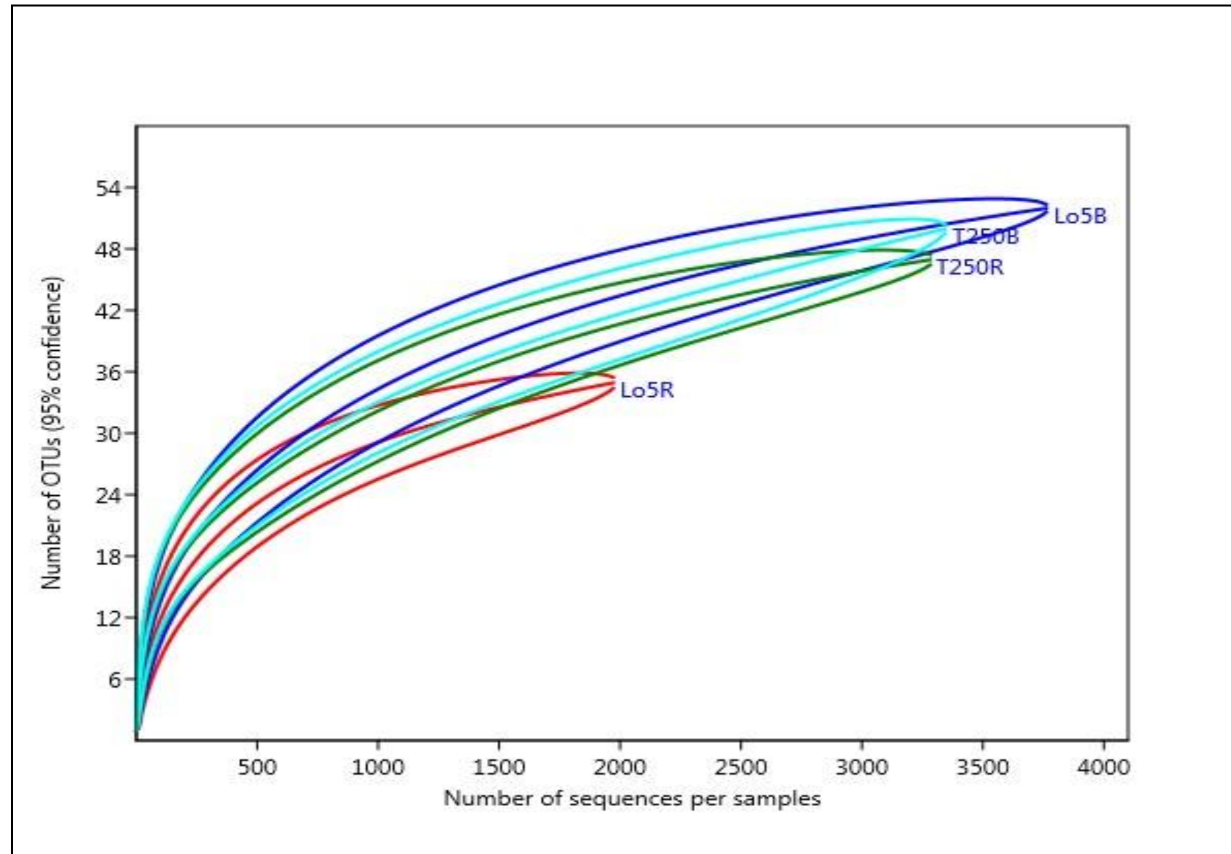
Uroz S, Buée M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2:281–288

World Reference Base for Soil Resources (2006) A framework for international classification, correlation and communication. World soil resources reports no. 103. FAO, Rome, **116 pp**

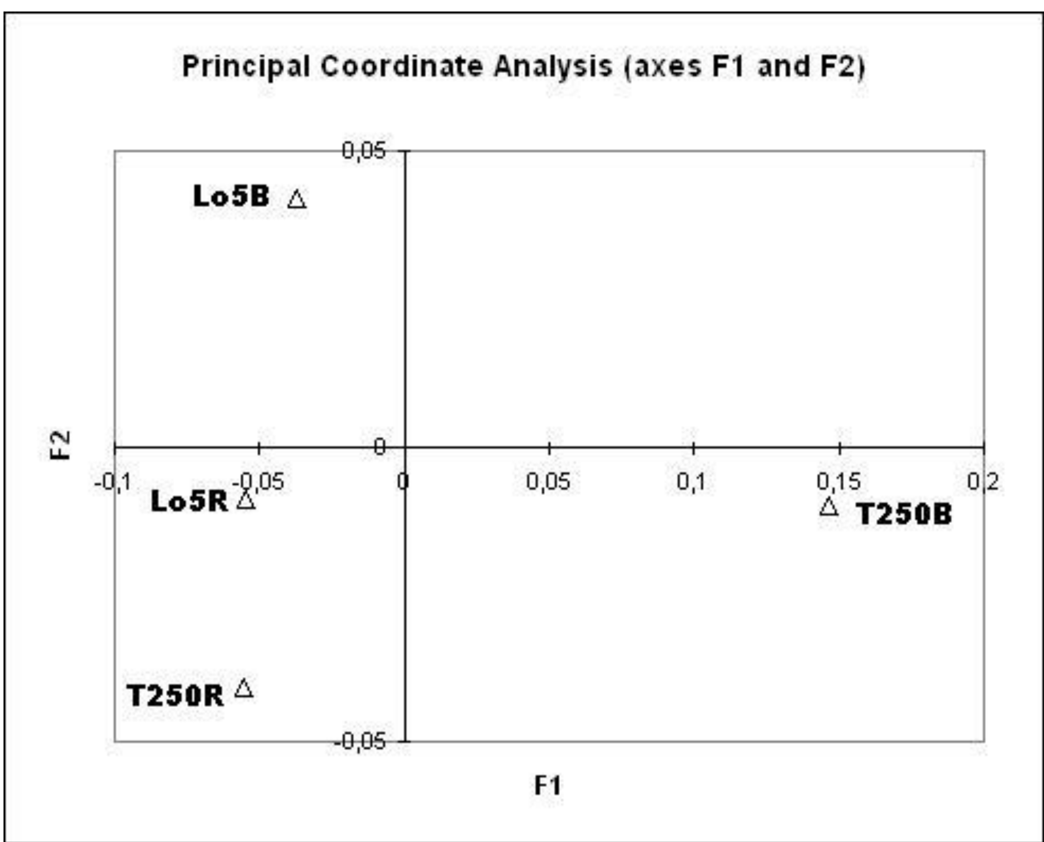
Xu XW, Huo YY, Wang CS, Oren A, Cui HL, Vedler E, Wu M (2011) *Pelagibacterium halotolerans* gen. nov., sp nov and *Pelagibacterium luteolum* sp nov., novel members of the family Hyphomicrobiaceae. Int J Syst Evol Microbiol 61:1817–1822

Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaver AK (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol 68:2198–2208

S-F 1 . Rarefaction analysis for molecular diversity of bacterial β-glucosidase-encoding genes in rhizosphere and bulk soil of Lo5 and T250 inbred maize lines. The letters R and B indicate rhizosphere and bulk soil respectively.



S-F 2. Principal Coordinate Analysis (PCoA) based on Raup and Crick probability similarity index between samples for bacetrial β-glucosidase gene sequences in rhizosphere and bulk soil of Lo5 and T250 maize varieties.



Chapter 4

"Seasonal variation and distribution of total and active microbial community of β-glucosidase encoding genes in forest soil"

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Seasonal variation and distribution of total and active microbial community of β-glucosidase encoding genes in forest soil

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Abstract

Organic matter decomposition plays an important role in the carbon cycle in terrestrial environments including the globally widespread coniferous forests. Cellulose degradation is very important in this respect because cellulose represents one of the most abundant polysaccharides in the plant litter. β-glucosidases complete the final step of cellulose hydrolysis by converting cellobiose to simple glucose molecules. Genetic potential and expression of β-glucosidases were studied in the forest floor of *Picea abies* forest in two contrasting seasons, late summer representing the peak of plant photosynthetic activity and late winter after a long period with no photosynthetic activity. Fungal and bacterial β-glucosidases belonging to the glycoside hydrolase gene families GH1 and GH3 were amplified from DNA and RNA and the amplicon pools were analysed. The pool of transcribed genes largely corresponded to the potential of the community encoded in the DNA, but multiple highly expressed genes were rare or even absent from the DNA gene pool. In fungi, Ascomycota and Basidiomycota and in bacteria the phyla Firmicutes, Actinobacteria, Proteobacteria, Acidobacteria and Deinococcus-thermus represented the major reservoirs of β-glucosidase genes and indicated that cellobiose utilization may be mediated by a highly diverse microbial community. Seasonality does have influence on potential diversity of β-glucosidase genes but intense changes occurred in transcription profile. In fungi, DNA derived communities were overlapping among two seasons or two horizons, especially in litter horizon during both seasons but transcribes showed distinct association with either L or H horizons in summer and winter. In bacteria, distribution of the abundant OTUs between summer and winter for each horizon was strict confinement of many OTUs in RNA derived community, either in summer or in winter, especially in humic horizon. Results indicate that rich communities of both bacteria and fungi express β-glucosidase. Even those genes showing low abundance may be functionally important as revealed by their high expression. The functional diversity in the studied ecosystem seasons clearly exhibited a seasonal pattern.

Introduction

Soil is one of the core reservoirs of the organic carbon compounds on Earth and hence, soil processes play a central role in the global C cycle. This is specifically the case in the coniferous forest soils that contain more than one third of all carbon stored in terrestrial ecosystems. Hence, understanding organic matter decomposition in the coniferous forest ecosystem is crucial for estimating global C fluxes and their potential future changes (Štursová et al., 2012; Baldrian et al., 2012). Dead plant biomass that accumulates on the forest surface is mostly composed of plant cell wall polymers, cellulose, hemicellulose and lignin. Cellulose, a glucose polymer linked by β-(1,4)-glycosidic bonds, is the most abundant polysaccharide in terrestrial environments and its degradation was the subject of focused research for decades. This research revealed that microorganism have the dominant role in this process in soils (Lynd et al., 2002; Baldrian and Valášková, 2008).

Enzymatic hydrolysis of cellulose typically requires the synergic action of three groups of hydrolytic enzymes: endoglucanases or endo-β-1,4-glucanases (EC 3.2.1.9.1), exoglucanases or cellobiohydrolases (EC 3.2.1.91) and β-1,4-glucosidases or cellobiases (EC 3.2.1.21). Among them, β-glucosidases complete the final step of cellulose hydrolysis by converting cellobiose to simple glucose molecules and deliver glucose for the central metabolism (Alef and Nannipieri, 1995). Its activity thus plays a vital role in the global-scale C cycle (Knight and Dick; 2004). β-glucosideses have also attracted considerable attention in recent years due to their important roles in diverse biotechnological processes such as bioethanol production, hydrolysis of isoflavone glucosidase, detoxification of cassava, elimination of bitter components from citrus products etc. (Singhania et al, 2013; Li et al, 2013).

Owing to its very large microbial diversity, soils are reservoir of C hydrolyzing activities (Nannipieri et al., 2012). However, in spite of their fundamental role in nature, the diversity of genes encoding for glycosidase hydrolases are still poorly understood. Recently, Bao et al (2012) obtained several β-glucosidases via metagenomic strategies and several sets of degenerate primers have been designed to analyze β-glucosidase gene diversity in soils (Kellner et al., 2010; Caňizares et al., 2011; Li et al 2013). None of these studies, however, used sufficient sequencing depth and only Li et al (2013) covered both fungi and bacteria using PCR-DGGE approach to analyse only potential diversity of β-glucosidase gene. Baldrian et al (2012) demonstrated the need to analyze the DNA and RNA gene pools to quantify the proportion of expressed genes and demonstrated this approach on fungal cellobiohydrolase *cbhI*. Cellulose is available in both litter and organic horizons of forest soils

(Šnajdr et al., 2011), hence similar cellulose degraders can be present and potentially active in both horizons. Though, litter contains higher amount of cellulose and likely supports higher diversity of cellulose degraders (Baldrian et al., 2012).

It was recently demonstrated that the seasonal variation of climatic conditions and consequent differences in tree physiology in the temperate and boreal zone forests are accompanied by the changes of microbial biomass content and composition of fungal and bacterial communities, which is a consequence of the limitation of the photosynthetic activity to the growing season with optimal temperature and light (Voříšková et al., 2014 and López Mondéjar et al., 2015). Some previous studies suggest that C allocation results of seasonal photosynthetic production can show profound influence on soil microbiota where subterranean C allocation through rhizodeposition mainly limited to growing season. Seasonality of enzyme processes and plant photosynthetic production were also demonstrated to largely affect microbial expression in temperate coniferous forests (Žifčáková et al., in press).

This study was performed in two contrasting seasons; late summer when plant photosynthetic activity was at peak and late winter (March) after a prolonged period with no photosynthate input. The aim was to demonstrate how the β-glucosidase genes and transcript pools of bacteria and fungi differ among horizons with different cellulose content and which members of the soil microbial community express the corresponding genes in the two different seasons. Kellner et al (2010) reported that β-glucosidase genes are expressed in forest soil by both the Ascomycota and Basidiomycota and Berlemont and Martiny (2013) reported that β-glucosidase genes are present in nearly all bacterial phyla. These facts demonstrate that both bacteria and fungi likely participate in the last step of enzymatic cellulose hydrolysis, but it is unclear what is the relative contribution of various taxa to this process.

Materials and Methods

Study site, sample collection

Study area was located in the highest altitudes (1170-1200 m) of the Bohemian Forest mountain range (Central Europe) and was covered by an unmanaged spruce (*Picea abies*) forest (49°02.64 N, 13°37.01 E). The site was previously studied with respect to the composition of total and active microbial communities in soils and the differences in gene expression in litter and soil among seasons (Baldrian et al. 2012, Žifčáková et al. in press). The mean annual temperature was 5 °C, and the mean

annual precipitation was 1000mm. Sampling was done in two contrasting seasons. These seasons were late summer (September) when plant photosynthetic activity was at peak and late winter (March) after a prolonged period with no photosynthate input and soil insulated by a deep snow cover. Soil temperature was around 15° C during September and 2° C in March. This study used the materials collected for the study of Žifčáková et al. (in press). Briefly, soil samples were collected from 6 sites, located 250 m from each other and eight soil cores (4.5 cm diameter) were collected from around the circumference of a 3m diameter circle. Litter horizon (L, 2-4 cm) and soil organic (humic) horizon (H, 3-6 cm) material were separately pooled. After removal of roots, L material was cut into 0.5 cm pieces and mixed; H material was passed through a 5-mm sterile mesh and mixed. A total of 24 samples were collected (6 sites × two seasons × two horizons). Aliquots for nucleic acids extraction were immediately frozen and stored at -80 °C, samples for chemical analysis and enzyme activity measurement were freeze-dried and stored at -45 °C. Enzyme assay was performed in soil homogenates (Štursová and Baldrian 2011).

Nucleic acid extraction and reverse transcription

Total DNA was extracted in triplicate from all samples using a modified Miller (Sagova-Mareckova et al., 2008) and cleaned with a Geneclean Turbo kit (MP Biomedicals). RNA was extracted using RNA PowerSoil Total Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) combined with the OneStep PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA, USA). Three soil aliquots (3 x 3 g of material) were extracted per samples. Triplicate of extracted RNA were pooled and RNA was purified using the RNA Clean & Concentration kit (ZymoResearch) on a column treated with DNase I (Fermentas) according to manufacturer’s instructions. These products were checked for quality (RIN number) and length distribution on an Agilent 2100 Bionalyser (Agilent Technologies). Approximately 1 µg of RNA was reverse transcribed using SuperScript III Reverse Transcriptase (Life Technologies) using random hexamer primers. M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and random hexamer primers. Samples were designated as LSD = Litter summer DNA, LWD = Litter winter DNA, HSD = humic summer DNA, HWD = humic winter DNA, LSR = Litter summer cDNA, LWR = Litter winter cDNA, HSR = humic summer cDNA, HWR = humic winter cDNA.

Primer design and tag-encoded amplicon sequencing

To identify β-glucosidase genes, two sets of degenerate primers were designed based on the sequences of β-glucosidase genes from a broad spectrum of soil fungi. All protein sequences annotated as β-glucosidase in the glycoside hydrolase (GH) families GH1 and GH3 in the CAZy database (http://www.cazy.org/) and the nucleotide counterparts of these protein sequences were retrieved from the GenBank (http://www.ncbi.nlm.nih.gov/). All nucleotide and protein sequences were de-replicated, multiple sequence alignments were conducted with MAFFT (Katoh et al., 2002) and alignments were curated manually. The primer pairs bglFGH1F/ bglFGH1R and bglFGH3F/ bglFGH3R were designed to amplify partial conserved fragments of GH1 and GH3 β-glucosidase genes from fungi, respectively (Table 1). All ambiguous positions were replaced with most common nucleotide at same positions from nucleotide sequence alignment. Designed primers were tested in SEED program (Větrovský et al., 2013) against available meta-transcriptomic data of same soil. The specificity of primers was tested using Primer-Blast tool from NCBI against GenBank dataset. The primer pairs βgluF2/βgluR4 (Cañizares et al., 2011) and GH3BF/GH3BR (Li et al., 2013) were used to amplify partial conserved fragments of GH1 and GH3 β-glucosidase genes from bacteria, respectively. Primers for tag-encoded sequencing contained in addition sample tag separated from primes by spacers. Spacer sequence were designed to have a trinucleotide, which was absent in all GenBank sequences at this positions to avoid overrepresentation of some target sequences (Parameswaran et al., 2007). Tagged primers were tested for self-dimmer, hetero-dimer and hairpin formation using the online tool OligoAnalyzer 3.1 (http://eu.idtdna.com/calc/analyzer).

All PCR amplification were performed in five replicate 25-μl PCR reactions. Reaction mixtures contained 1 μl of template DNA/cDNA, 2.5 μl of 10× polymerase buffer, 1 μl of each primer (0.01 mM), 1.5 μl of 10 mg ml^{-1} of BSA, 1 μl of PCR Nucleotide Mix (10mM) and 0.75 μl of polymerase (2U $μl^{-1}$; Pfu DNA polymerase:DyNAZyme II DNA Polymerase, 1:24). Cycling conditions were 94 °C for 5 min; 35 cycles of 94 °C for 45 s, 53 °C for 45 s, 72 °C for 1 min, followed by 72 °C for 10 min for the primers bglFGH1F/ bglFGH1R; 94 °C for 5 min; 35 cycles of 94 °C for 45 s, 50 °C for 45s, 72 °C for 1 min, followed by 72 °C for 10 min for the primers bglFGH3F/ bglFGH3R; 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min, followed by 72 °C for 10 min for the primers βgluF2/βgluR4 and 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min, followed by 72 °C for 10 min for the primers BGH3BF/BGH3BR. PCR products were separated by electrophoresis and gel purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). DNA was quantified using a Qubit® 2.0 fluorometer (Invitrogen, USA), an equimolar mix of PCR products from all samples was made for each primer

pair and the pooled products were mixed and subjected to paired-end sequencing (2 x 250 bp) on the Illumina Miseq sequencer.

Sequence analysis and statistics

Paired reads were assembled, quality-filtered and analyzed using the pipeline SEED 1.2.1 (Větrovský and Baldrian, 2013). Briefly, chimeric sequences were detected using UCHIME algorithm and removed from dataset. Sequences were then clustered into operational taxonomic units (OTUs) at a 97% sequence identity threshold using the UPARSE algorithm, implemented in USEARCH v7.01.1001 and consensus sequences were constructed for all OTUs (Větrovský and Baldrian, 2013). For identification and taxonomic assignment, representative sequences were retrieved from the CAZy database (http://mothra.ornl.gov/cgi-bin/cat/cat_v2.cgi?tab=ORTHOLOGS, www.cazy.org) and the JGI (Joint Genome Institute) (http://genome.jgi.doe.gov/programs/fungi/GE_Fungi.jsf#diversity) using 10^{-4} E value threshold. Sequences obtained in this study, which showed low similarity to β-glucosidase genes of fungi, or bacteria were discarded.

Rarefaction and diversity analyses on OTUs were performed on resampled datasets with the same number of sequences randomly selected from all samples (Table 2). Richness, diversity indices and Chao-1 estimates were calculated using SEED 1.2.1 (Větrovský and Baldrian, 2013). ANOVA followed by the Tukey *post hoc* test was also performed on diversity indices to evaluate statistical differences among all different treatments using the R Statistics Environment (R Development CoreTeam, 2008). Global singletons were excluded from further analyses. The DNA/RNA ratio was calculated as the sum of sequences derived from DNA divided by the sum of all sequences, Litter/Soil and Summer/Winter ratios were also calculated in the same way. Nucleotide sequence of OTUs with an abundance over 0.3% were also translated into amino acid sequences using BioEdit 7.2.5 (Ibis BioScience, Carlsbad, CA) and curated manually.

Phylogenetic assignment to microbial phyla was also estimated based on best hits, by dividing the number of sequences belonging to each phylogenetic group by the total number of sequences in given samples ANOSIM was performed based on Bray-Curtis similarity distances using abundance data of all OTUs or clusters to assess the significance of difference among β-glucosidase gene pools from different treatments. The R values in ANOSIM ranges from 0 to 1, where $R > 0.7$ indicates significant difference, $R < 0.25$ high similarity and $0.7 < R > 0.25$ moderate distribution. PCoA (Principal

Coordinate Analysis) was also performed based on Bray-Curtis similarity distance on abundance data of all OTUs with > 0.3% abundance to visualize differences among β-glucosidase gene pools of different treatments. ANOSIM and PCoA were calculated using PAST 3.06 (Hammer et al. 2001).

Phylogenetic tree analysis were performed on nucleotide and translated amino acid sequences of OTUs with abundance over > 0.3%. Sequences were aligned using MAFFT (Katoh et al., 2002) to all homologous sequences retrieved from CAZy database (http://www.cazy.org/). Maximum-Likelihood (ML) trees were generated by MEGA 6 (Tamura et al., 2013) using JTT model (Jones et al., 1992) for protein sequences. Robustness of the tree topology was tested by bootstrap analysis (100 replicates).

Results

Soil chemical properties and enzyme activity

Soils were characterized by high content of organic matter and low pH. The chemical properties of litter and soil differed dramatically, with the litter horizon containing significantly more organic matter, as well as nutrients (C, N, and P) and exhibiting slightly but significantly higher pH and moisture content (Žifčáková et al., in Press). β-glucosidase activity was significantly higher in litter than in soil but there was no significant differences observed for seasonality in each horizon (Supplementary Figure 1).

Metagenomic potential and expression of GH1 β-glucosidase genes

For bacteria, Illumina Miseq sequencing yielded a total of 563 763 sequences with a total number of 14 689 OTUs. Shannon index was observed between 2.25-3.24 and species richness 11.17-36 (Supplementary Table 1a). RNA derived community from soil during the winter was significantly less diverse than the other RNA and DNA derived communities (Supplementary Table 1a). Evenness was found between 0.89-0.97 (Supplementary Table 1a). DNA and RNA derived communities were quite evenly distributed (Supplementary Table 1a). Chao 1 estimator was observed within 31.97-133.9 with lowest OTUs value detected for RNA derived community from soil during winter and Chao 1 index was significantly higher in DNA derived samples than those of RNA derived samples

(Supplementary Table 1a). Approximately 57% and 40% of DNA sequences were transcribed in soil during summer and winter respectively, while 62% and 56% of DNA sequences were transcribed in litter during summer and winter, respectively (Table 3). No significant differences observed during transcription of bacterial β-glucosidase gene sequences between two seasons or different horizons (Table 3). ANOSIM and PCoA analysis based on Bray-Curtis similarity distance showed significant differences ($R > 0.7$) in GH1 bacterial β-glucosidase pool composition between RNA and DNA derived communities of litter and soil. (Supplementary Table 2a & Supplementary Figure 2).

We observed that for 90-95% of OTUs, all 5 top hits belonged to the same phylum and 110 OTUs showed > 90% similarity with an identified reference sequence. Actinobacteria followed by Proteobacteria were the most abundant phylum of carrying GH1 bacterial β-glucosidase genes but sequences belonging to Bacteriodetes, Chloroflexi, Deinococcus-Thermus, Dictiyoglomy, Firmicutes, Nitrospiare, Spirochaetes, Thermotogae and Verrumicrobia were also detected (Figure 1a). Only 1-3 % of sequences originating from the DNA pool were unassigned while this number was 18%-35% for the RNA-derived sequences (Figure 1a). In bacteria, actinobacterial OTUs were overlapping in summer and winter for both horizons but low abundant phyla showed firm distribution in DNA and RNA communities for both horizon during both seasons (Figure 2). Bacterial OTUs were more horizon specific, especially for Soil organic horizon (Figure 2). Phylogenetic tress were constructed using peptide sequences allowed coarse taxonomic placement of producers for approximately 72% and 19% of most abundant bacterial β-glucosidase genes from GH1 family clustered into actinobacteria and proteobacteria, respectively (Supplementary Figure 4, Supplementary Table 3

For Fungi, Illumina Miseq sequencing yielded a total of 11 443 sequences with a total number 1166 fungal OTUs. Shannon index was found within 0.91-3.26 range and species richness between 5.67-32.57 (Supplementary Table 1c). RNA derived communities were significantly less diversified than those of DNA derived communities (Supplementary Table 1c). Evenness was found between 0.55-0.94 and RNA communities were significantly uneven between two horizons, while DNA communities were fairly even (Supplementary Table 1c). Chao 1 estimator was observed within 10.08-90.65 and it was significantly higher in DNA derived communities than those of RNA derived communities (Supplementary Table c). Approximately, 24% and 16% of DNA sequences were transcribed in soil during summer and winter, respectively, though 23% and 30% of DNA sequences were transcribed in litter during summer and winter, respectively (Table 3). No significant differences observed during transcription of fungal β-glucosidase gene sequences (Table 3). ANOSIM results

showed significant dissimilarity (R > 0.7) between DNA and RNA derived communities' compositions (Supplementary Table 2c). PCoA results showed that most abundant OTUs from RNA derived community of winter litter is significantly dissimilar to the other samples (Supplementary Figure 3).

We observed that for 100% of OTUs, all 5 top hits belonged to the same phylum. 13 OTUs showing 100 % similarity and 23 OTUs showing > 90 % similarity with identified reference sequences. Ascomycota was only identified phylum who was carrying GH1 fungal β-glucosidase genes but up to 50% of DNA derived communities is unassigned (Figure 1c). Approximately 92-97% of RNA derived communities were belonging to Ascomycota and only 2-3% of genes were transcribed from unassigned sequences (Figure 1c). Fungal β-glucosidase showed distinct distribution of most abundant gene pools among different seasons and horizons (Figure 4). Most abundant OTUs were overlapping each other in DNA communities but distinct distribution was observed in RNA community in both horizons during summer and winter (Figure 4). Phylogenetic tress of fungal β-glucosidase genes showed that all most all abundant sequences were assigned as Ascomycota (Supplementary Figure 5, Supplementary Table 4).

Metagenomic potential and expression of GH3 β-glucosidase genes

For bacteria, Illumina Miseq sequencing yielded a total of 1 17 66 416 sequences with a total number of 63 325 OTUs. The Shannon index was observed within 2.42-3.18 range and species richness was 20.67-32.83 (Supplementary Table 1b). DNA derived communities were highly diverse than those of RNA derived communities (Supplementary Table 1b). Evenness was found between 0.67-0.95 (Supplementary Table 1b). RNA derived communities were significantly unevenly distributed in both horizons and also in both seasons, whether DNA derived communities were evenly distributed between two seasons for each horizon but significant differences observed between two horizon (Supplementary Table 1b). Chao 1 estimator was detected within range of 49.86-207.22 and it was significantly higher in DNA derive communities than those of RNA derived communities (Supplementary Table 1b). Approximately 40% and 52% DNA sequences were transcribed in soil during summer and winter respectively while 30 % and 19 % of DNA sequences were transcribed in litter during summer and winter, respectively (Table 3). Significantly, higher number of OTUs transcribed in soil during winter than summer and also than litter in both seasons (Table 3). ANOSIM results showed moderate distribution between DNA and RNA community but DNA community of litter in summer and winter was significantly differ from DNA community of winter humic horizon

(R > 0.7) (Supplementary Table 2b). PCoA results showed that DNA and RNA communities from litter and summer were clustering separately from each other (Supplementary Figure 2).

We observed that for 90% of OTUs, all 5 top hits belonged to the same phylum. Only 11 OTUs showing ≥ 90 % similarity with identified reference sequences. Firmicutes was most abundant identified phyla, carrying β-glucosidase encoding genes. β-glucosidase encoding genes of Proteobacteria, Actinobacteria and Acidobacteria were also detected and 5% of β-glucosidase genes of low abundant phyla were also found. Up to 50% of DNA derived community was unassigned and approximately 70% of genes were transcribed by these unknown organisms (Figure 1b). OTU 1 (*Paenibacillus sp.*) and OTU 5 (*Acidobacterium sp.*) were highly abundant in DNA and RNA community in both horizons during summer and winter (Figure 3). Phylogenetic tree showed that 69% of most abundant β-glucosidase sequences clustered into firmicutes. However 11%, 8% and 5% of sequences related to acidobacteria, proteobacteria and actinobacteria, respectively (Supplementary Figure 4, Supplementary Table 5).

For Fungi, Illumina Miseq sequencing yielded a total of 6 34 236 sequences with a total number 7178 fungal OTUs. The Shannon index was found within 1.67-2.9 range and species richness was between 7.17-26.67 (Supplementary Table 1d). DNA derived communities were highly diverse than those of RNA derived communities (Supplementary Table 1d). Significant differences were observed between two horizons and between both seasons in RNA community but in DNA community, significant differences only observed between horizons (Supplementary Table 1d). Evenness was found between 0.71-0.91 but DNA and RNA derived communities of litter in winter was only significantly uneven than the other samples (Supplementary Table 1d). Chao 1 estimator was observed between 12.58-53.24 ranges (Supplementary Table 1d). Surprisingly, highest number OTUs were detected in RNA derived community of litter in summer than the other samples (Supplementary Table 1d). Significant differences were only observed between summer and winter for RNA derived communities of litter (Supplementary Table 1d). Approximately 33% and 23% DNA sequences were transcribed in soil during summer and winter respectively f while 69 % and 44 % of DNA sequences were transcribed in litter in summer and winter, respectively (Table 3). Significantly higher number of OTUs transcribed in litter than soil (Table 3). ANOSIM results showed that significant similarity or moderate distribution between DNA and RNA derived communities of summer and winter humic horizon although significant dissimilarity (R > 0.7) were observed in DNA and RNA derived communities of summer and winter litter (Supplementary Table 2d). Even, β-glucosidase gene diversity was expressively different between RNA derived communities of both horizons

(Supplementary Table 2d). PCoA results showed that DNA derived communities of summer and winter were clustering together for both horizons whereas RNA derived communities of both horizons were clustering separately for both seasons (Supplementary Figure 3).

We observed that for 90-95 % of OTUs, all 5 top hits belonged to the same phylum. 7 clusters showing > 97% and 170 clusters showing ≥ 90 similarity with identified reference sequences. Ascomycota and Basidiomycota being most abundant phylum who carrying fungal β-glucosidase genes from GH3 family but few sequences belonging to Zygomycota were also detected (Figure 1d). Approximately 93% of DNA sequences belonging to Ascomycota and only very, less number of sequences belonging to Basidiomycota and Zygomycota but approximately 60 % of RNA derived communities were transcribed by Basidiomycota (Figure 1d). Still, there were approximately 20% of sequences were unknown (Figure 1d). Distinctive distribution was observed for most abundant gene pools between both horizons in different seasons (Figure 5). Phylogenetic trees showed bristly taxonomic assignment of producers for approximately 79 % and 14 % of most abundant fungal β-glucosidase genes of GH3 family clustered into ascomycota and basidiomycota, respectively (Supplementary Figure 5, Supplementary Table 6).

Discussion

β-glucosidase activity was significantly higher in litter than the organic soil which shows more rapid organic matter transformation in litter than in soil, agreed with previous reports (Baldrian et al., 2012). Forest soils represent an environment that exhibits distinct and sharp vertical stratification. The ultimate cause is likely to be the decrease in organic matter with soil depth as a result of the accumulation of litter on the soil surface (Voříšková et al., 2014) and β-glucosidase activity is known to increase with increasing organic matter content (William and Jochem, 2006). There were no seasonal differences in enzyme activities, which could be due to limitations of substrate supply from cellulases to β-glucosidase by releasing cellobiose from cellulose in forest soil. Our results are in agreement with previous studies (Baldrian et al., 2013; Žifčáková et al., 2015); reported that seasonality does not have significant effect on extracellular enzyme activities.

In this study, first time we have studied phylogenetic distribution of single functional gene in bacteria and fungi, involved in cellulose decomposition using enough in depth sequencing in forest soils. Forest topsoils have been previously demonstrated to exhibit vertical stratification of organic matter and organic matter degraders resulting of different chemical composition (Baldrian et al., 2012). RNA derived community were less diverse and less even than those of DNA derived community of fungal β-glucosidase encoding gene, repressing that only selected part of potential community could be

metabolically active at given time. Surprisingly, diversity index and chao estimator was significantly higher in RNA derived community of litter during summer for β-glucosidase encoding gene from GH3 family, because many fungi harbour more than one β-glucosidase encoding genes (Eichlerová et al., 2015).

We found that the Ascomycota was only a phylum which carrying β-glucosidase encoding genes from GH1 family in studied environment. In GH3 family, ascomycota was clearly dominated in DNA derived community but Basidiomycota (typical saprotrophs) was clearly more active during expression of genes except in soil organic horizon during winter season. This showed importance of low-abundance species in cellulose hydrolysis and other soil functions. This is due to higher activity of saprotrophic fungi in litter and dominance of ectomycorrhizal fungi in deeper soils (Lindahl et al., 2007; Edward and Zak, 2010). It was previously reported that saprotrophic fungi are mainly associated with litter decomposition (Voříšková and Baldrian, 2013). The high activity of Ascomycota in soil in winter which is due to high abundance of fungi in the rhizosphere than the bulk soil (Tuner et al., 2013) and seasonality of root process take place in soil and not in litter. Žifčáková et al., 2015 suggested that rhizodeposition of photosynthetically fixed C could explain the decrease of fungal activity in soil organic horizon during winter due to superiority of ECM fungi in system. In total, approximately 60% and 42% of fungal β-glucosidase encoding gene sequences present in DNA were being transcribed in litter during summer and winter, respectively; while only 30% and 20 % of DNA sequences were being transcribed in soil organic horizon during summer and winter, respectively. This is clearly indicating the dominant role of fungi in litter decomposition and decrease of cellulolytic fungi with soil depth.

In RNA derived community of fungal β-glucosidase encoding gene from GH3 family, OTU0 was highly dominant; especially in litter during summer. From GH1 family, OTU0 was also significantly rich in RNA derived community of fungal β-glucosidase encoding gene; especially in humic horizon during winter. These showed distinctive distribution of most abundant β-glucosidase gene pools from fungal community between both horizons in different seasons and also between DNA and RNA for both, GH1 and GH3 families. In both families, DNA derived communities are more or less overlapping among two seasons or two horizons, especially in litter horizon during both seasons. However RNA derived communities showed firm confinement between two horizons and between summer and winter for both horizons (especially in litter).

Actinobacteria and Proteobacteria were being most abundant phyla, carrying β-glucosidase encoding genes in DNA community of GH1 family but 30-40% of transcripts were mainly origin of low abundant phyla such as Deinococcus-thermus, Bacteroidetes, Firmicutes, Thermotogae and unknown organisms. For GH3 family, firmicutes was most abundant phyla, carrying β-glucosidase encoding genes followed by actinobacteria, proteobacteria and acidobacteria in both DNA and RNA derived communities but up to 40-50 % of sequences are still unknown due to lack of sequence information in public data set. Identified cellulolytic bacterial taxa from this study were also previously recorded in agricultural and forest soils (Wirth and Ulrich, 2002; Haichar et al., 2007; Ulrich et al., 2008; Schellenberger et al., 2010, Štursová et al., 2012; Baldrian et al., 2012). Berlemont and Martiny (2013) reported that β-glucosidase genes are present in nearly all bacterium phyla which showing importance of bacteria in cellulose degradation. In Actinobacteria, *Streptomyces* was most abundant genera, carrying and transcribing β-glucosidase encoding genes. Genome sequencing of *Streptomyces* (de Oliveira et al., 2014) and isolation culturing (Perez-Pons et al., 1994) showed that *Streptomyces* carrying β-glucosidase encoding genes and involved in cellulose degradation. There was distinct distribution of most abundant β-glucosidase gene pools from fungal community between both horizons but there was minor overlapping among taxa in DNA derived community. The distribution of the abundant OTUs between summer and winter for each horizon was strict confinement of many OTUs in RNA derived community, either in summer or in winter, especially in humic horizon indicating importance of bacteria in soil organic horizon during degradation process. Transcripts were origin from different bacterial taxa and genera in litter and soil organic horizon during summer and winter, because of differences in nutrient availability and the presence of root-associated bacteria due to rhizodeposition of plants in soil (O'Brien et al., 2005; Lindahl et al., 2007; Šnajdr et al., 2008).

In total more or less only 50% to 60% of DNA derived β-glucosidase gene pool were transcribed in both Fungi and bacteria and only specific or selected microbial community was metabolically active and involved in cellulose degradation in forest soil. Low abundant taxa have important role in cellulose hydrolysis process in forest soil, because of their high activity during transcription. These data showed that the DNA sequencing approaches miss a significant and functionally relevant part of microbial communities and our current knowledge on largely based on this approach is incomplete (Baldrian et al., 2012). Many of fungal and bacterial species were carrying β-glucosidase encoding genes from both GH1 and GH3 families. This is suggesting that many bacterial and fungal species contain more than one β-glucosidase genes but they are quite different from each other in sequence similarity. In fungi, ascomycota and basidiomycota and in bacteria, actinobacteria, proteobacteria, firmicutes and acidobacteria were abundant phyla, harbour and transcribing β-glucosidase genes in

forest soil. These facts make both bacteria and fungi suitable tracers involved in enzymatic cellulose hydrolysis and C cycle. The consensus sequences of bacterial and fungal β-glucosidase genes derived from this study differed and more diverse from previously published sequences which were mainly based on cloning and sequencing of DGGE bands (Kellner et al., 2010; Caňizares et al., 2011; Li et al 2013). Hence, the depth of environmental amplicon sequencing may contribute to better evaluation of targeted functional gene diversity. Seasonality of plant photosynthetic production is significantly influencing diversity and expression of β-glucosidase producing microorganism in forest litter. Contrary to above data, seasons did not have any kind of effect on β-glucosidase activity which suggesting that analysis of gene expression at protein level is needed to better understand the link between diversity of β-glucosidase encoding gene and expressed β-glucosidase protein.

Conclusion

Our results confirmed that seasonality is likely key driver of changes in β-glucosidase encoding gene diversity and their expression. Fungi and bacteria both are important traces harbour β-glucosidase encoding gene and involved in cellulose degradation. Diversity and distribution of functional genes mainly regulating important biogeochemical processes and several low abundant bacterial and fungal taxa highly expressing β-glucosidase gene, showing importance of these species in organic matter decomposition. Future research is needed to characterize expressed β-glucosidase protein using proteomic approach to better explain link between diversity of encoding genes and their expression.

References

Alef K and Nannipieri P (1995) Methods in Applied Soil Microbiology and Biochemistry. ISBN: 978-0-12-51380-6

Baldrian P & Valášková V (2008) Degradation of cellulose by basidiomycetous fungi. FEMS Microbiol Rev 32: 501–521.

Baldrian P, Kolařík M, Štursová M, Kopecky J, Valášková V, Větrovsky T, Žifčáková L, Anajdr J, Rídl J, Vlček C, Vaříšková J (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. The ISME Journal 6: 248-258

Baldrian, P., Šnajdr, J., Merhautova, V., Dobiašova, P., Cajthaml, T., Valaškova, V. (2013) Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biol Biochem 56: 60-68

Bao, L., Huang, Q., Chang, L., Sun, Q., Zhou, J., Lu, H., 2012. Cloning and characterization of two beta-glucosidase/xylosidase enzymes from yak rumen metagenome. Appl. Biochem. Biotechnol. 166, 72–86.

Berlemont R, Martini CA (2013) Phylogenetic distribution of potential cellulases in bacteria. Applied and Environmental Microbiology 79: 1545-1554

Bray JR and Curtis JT (1957) An ordination of upland forest communities of southern Wisconsin. Ecological Monographs 27:325-349

Cañizares R, Benitez E, Ogunseitan OA (2011) Molecular analyses of beta-glucosidase diversity and function in soil. European Journal of Biology 47: 1-8

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117-143

de Oliveira LG, Tormet Gonzalez GD, Samborsky M, Marcon J, Araujo WL, de Azevedo JL (2014) Genome sequence of Streptomyces wadayamensis strain A23, an endophytic actinobacterium from Citrus reticulata. Genome Announc. 2(4):e00625-14. doi:10.1128/genomeA.00625-14

Edwards IP, Zak DR. (2010). Phylogenetic similarity and structure of Agaricomycotina communities across a forested landscape. Mol Ecol 19: 1469–1482

Eichlerová I, Homolka L, Žifčáková L, Lisá L, Dobiášová P, Baldrian P (2015) Enzymatic systems involved in decomposition refelects the ecology and taxonomy of saprotrophic fungi. Fungal Ecology 13: 10-22

Haichar FEZ, Achouak W, Christen R, Heulin T, Marol C, Marais MF, Mougel C, Ranjard L, Balesdent J & Berge O (2007) Identification of cellulolytic bacteria in soil by stable isotope probing. Environ Microbiol 9: 625–634

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistic software package for education and data analysis. Palaeontol Electron 4:1–9

Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Computer Applications in the Biosciences 8: 275-282

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequences alignment based on fat Fourier transform. Nucleic Acid Research 30: 3059-3066

Kellner H, Vandenbol M (2010) Fungi unearthed: Transcripts encoding lignocellulolytic and

chitinolytic enzymes in forest soil. PLoS ONE 5(6): e10971

Knight TR, Dick RP (2004) Differentiating microbial and stabilized β- glucosidase activity relative to soil quality. Soil Biol Biochem 36: 2089–2096

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J et al. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol 173: 611–620

Lombard V, Ramulu HG, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42: 490–495

López-Mondéjar R, Voříšková J, Větrovský T, Baldrian P (2015). The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. Soil Biol Biochem 87: 43-50

Lynd LR, Weimer PJ, van Zyl WH & Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66: 506–577.

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B (2012) Soil enzymology: classical and molecular approaches. Biol Fertil Soils 48: 743-762

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. (2005). Fungal community analysis by large-scale sequencing of environmental samples. Appl Environ Microbiol 71: 5544–5550

Parameswaran P, Jalili R, Tao L, Shokralla S, Gharizadeh B, Ronaghi M et al. (2007). A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing. Nucleic Acids Res 35: e130

Perez-Pons JP, Cayetano A, Rebordosa X, Querol E (1994) A β-glucosidase gene (bgl3) from *Sterptomyces sp.* Strains QM-B814 molecular cloning, nucleotide sequence, purification and

charachterization of the encoded enzyme, a new member of family 1 glycosyl hydrolases. Eur. J. Biochem. 233: 557-565

Schellenberger S, Kolb S & Drake HL (2010) Metabolic responses of novel cellulolytic and saccharolytic agricultural soil Bacteria to oxygen. Environ Microbiol 12: 845–861

Singhania, R.R., et al. Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. Bioresour. Technol. (2012), http://dx.doi.org/10.1016/j.biortech.2012.09.012

Šnajdr, J., Valaškova, V., Merhautova, V., Herinkova, J., Cajthaml, T., Baldrian, P. (2008) Spatial variability of enzyme activities and microbial biomass in the upper layers of *Quercus petraea* forest soil. Soil Biol Biochem 40: 2068-2075.

Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P (2012) Cellulose utilization in forest litter and soil: Identification of bacterial cellulose decomposers. FEMS Microbiol Ecol 80: 735-746

Turner, T.R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D. et al. (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME J **7:** 2248-2258

Tamura K, Stecher G, Peterson D, Filipski A, Kumar s (2013) MEGA 6: Molecular Evolutionary Genetic Analysis Version 6.0. Molecular Biology and Evolution 30: 275-2729

Ulrich A, Klimke G, Wirth S (2008) Diversity and activity of cellulose-decomposing bacteria, isolated from a sandy and a loamy soil after long-term manure application. Microb Ecol 55: 512–522

Vaříšková J, Brabcová V, Cajthaml T, Baldrian P (2014) Seasonal dynamics of fungal communities in a temperate Oak forest soil. New Phytol 201: 269-278

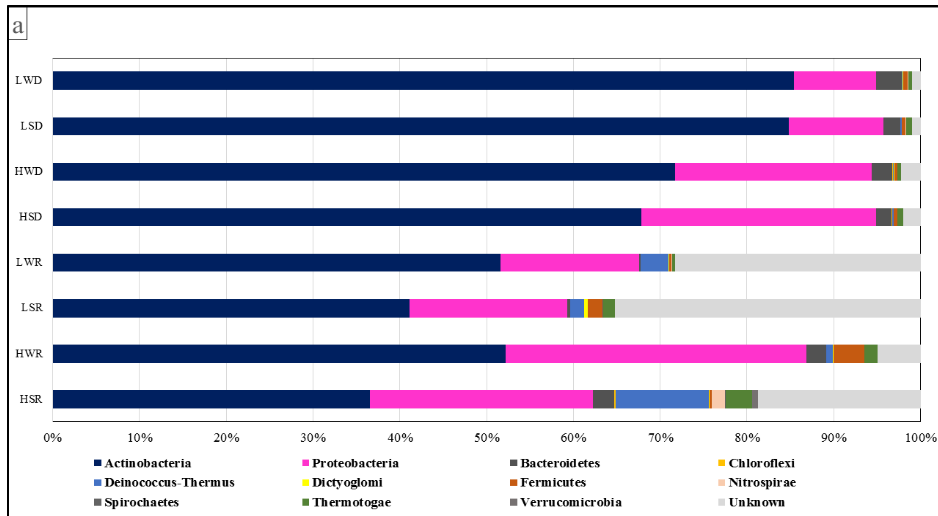
Větrovsky, T., Baldrian, P. (2013) Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED. Biol Fertil Soils 49:1027-1037

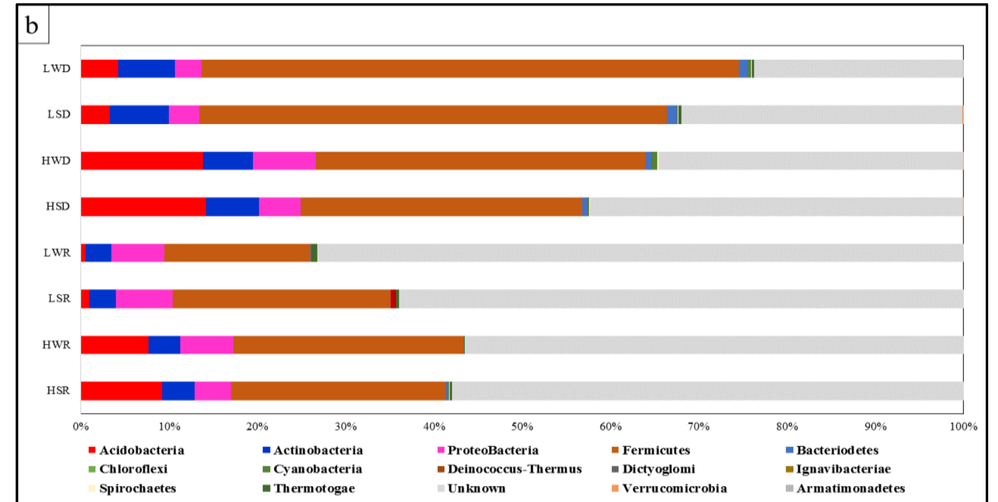
Williams, C., and Jochem, F.J. (2006) Ectoenzyme kinetics in Florida Bay: Implications for bacterial carbon source and nutrient status. Hydrobiologia 569: 113–127

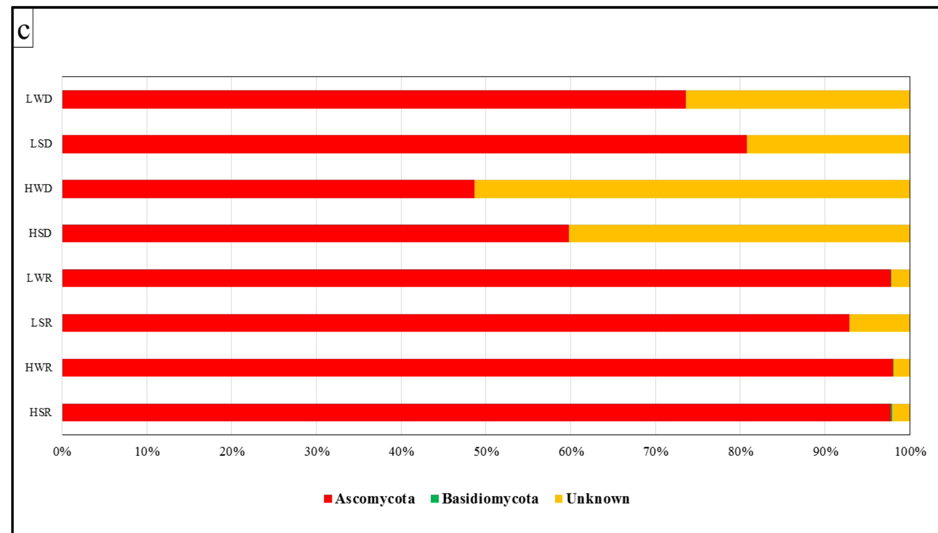
Wirth S & Ulrich A (2002) Cellulose-degrading potentials and phylogenetic classification of carboxymethyl-cellulosdecomposing bacteria isolated from soil. Syst Appl Microbio 25: 584–591

Žifčáková L, Větrovsky T, Adina CH, Baldrian P (2015) Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Env. Microbiol. In Press

Figure 1. Taxonomic assignment of β-glucosidase genes and transcripts from the *Picea abies* forest litter and soil in summer and winter. a) GH1 family bacteria β-glucosidase OTUs, b) GH3 family bacteria β-glucosidase OTUs, c) GH1 family fungal β-glucosidase OTUs and d) GH3 family fungal β-glucosidase OTUs.







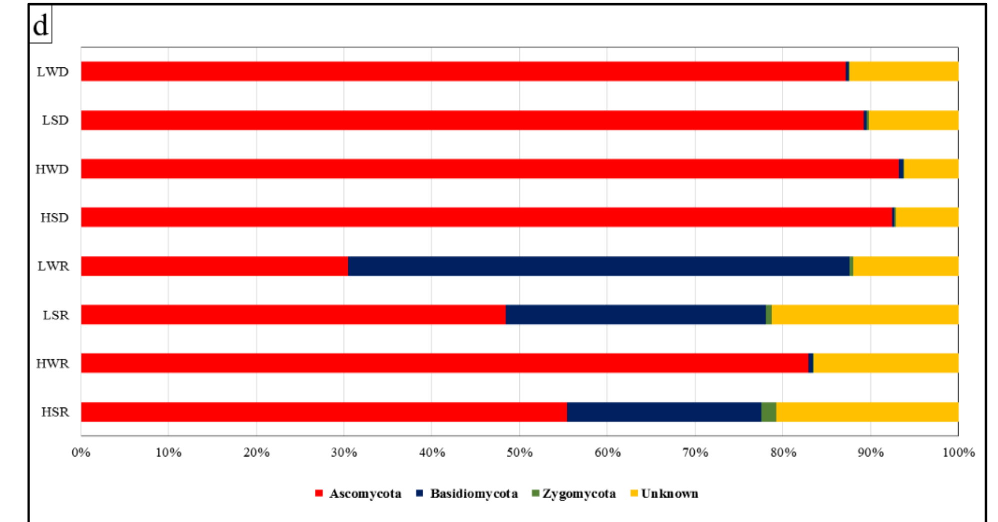


Figure 2. Distribution of major GH1 family bacterial β-glucosidase gene OTUs from *Picea abies* forest litter and soil among horizons and seasons. a) between litter and H horizon and between DNA and RNA, b) between summer and winter and between DNA and RNA for litter horizon and c) between summer and winter and between DNA and RNA for soil.

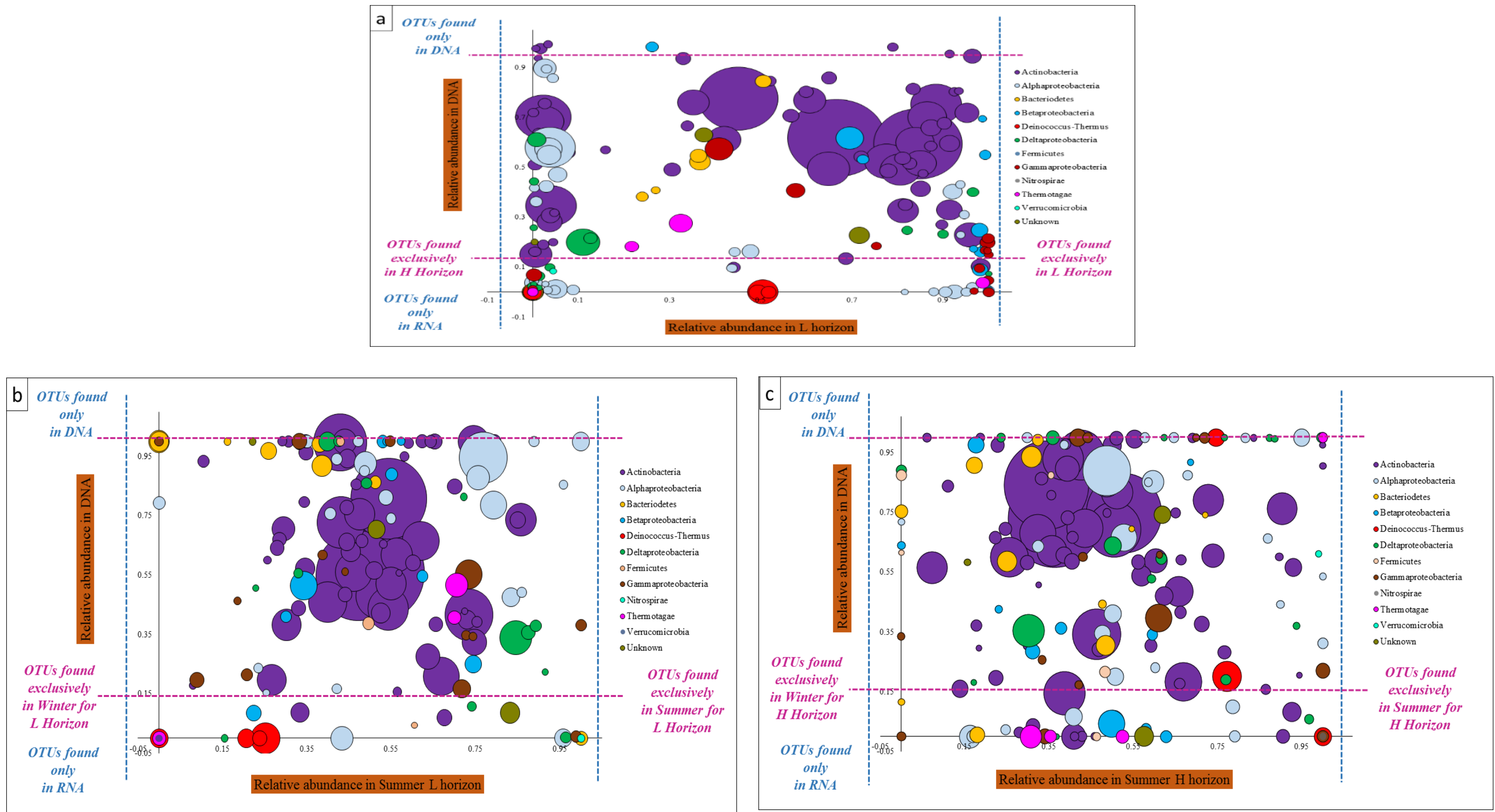
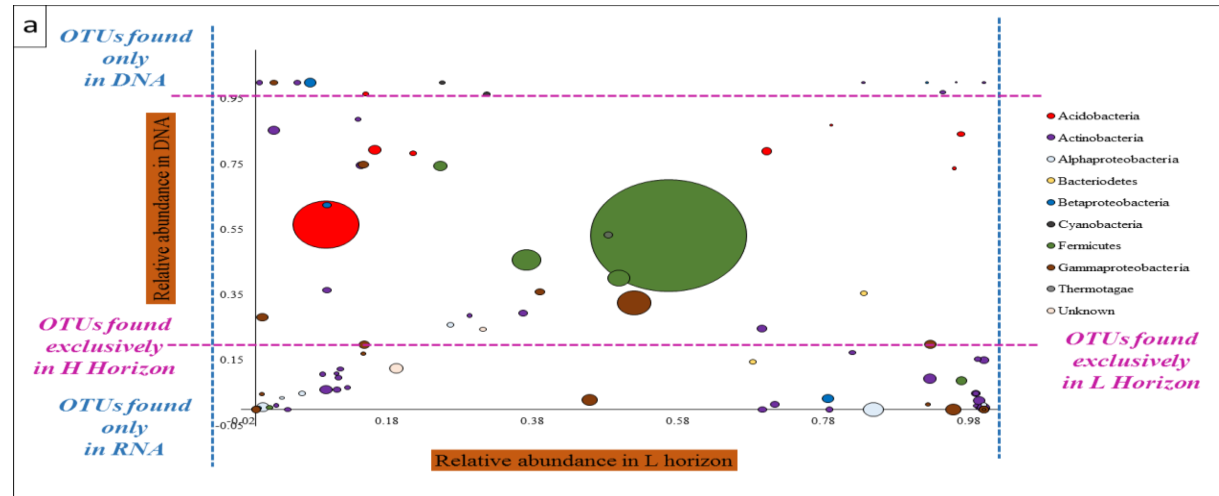
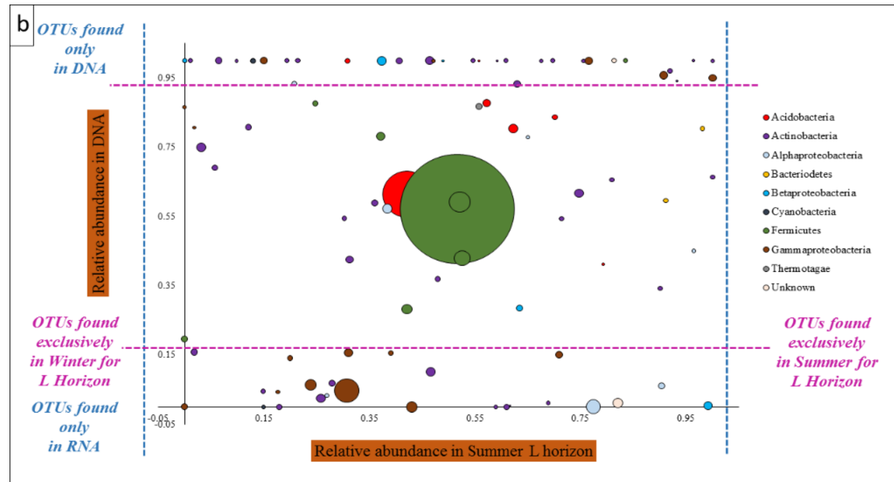


Figure 3. Distribution of major GH3 family bacterial β-glucosidase gene OTUs from *Picea abies* forest litter and soil among horizons and seasons. a) between litter and H horizon and between DNA and RNA, b) between summer and winter and between DNA and RNA for litter horizon and c) between summer and winter and between DNA and RNA for soil.





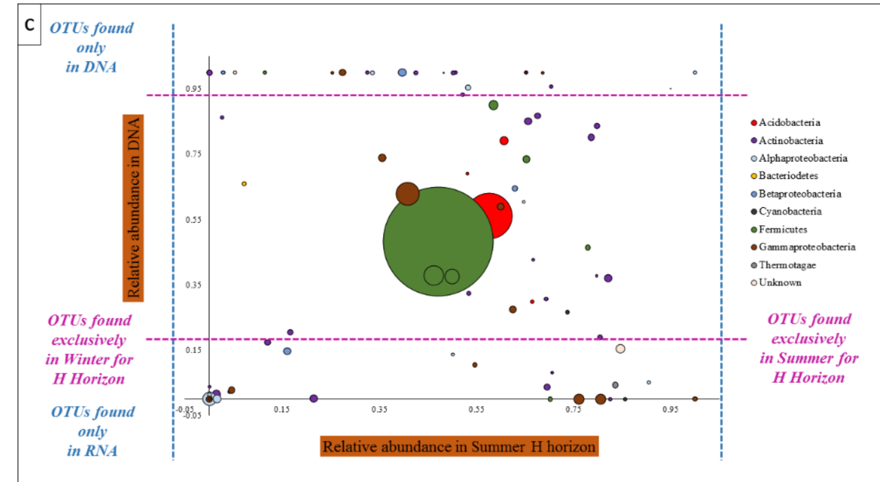
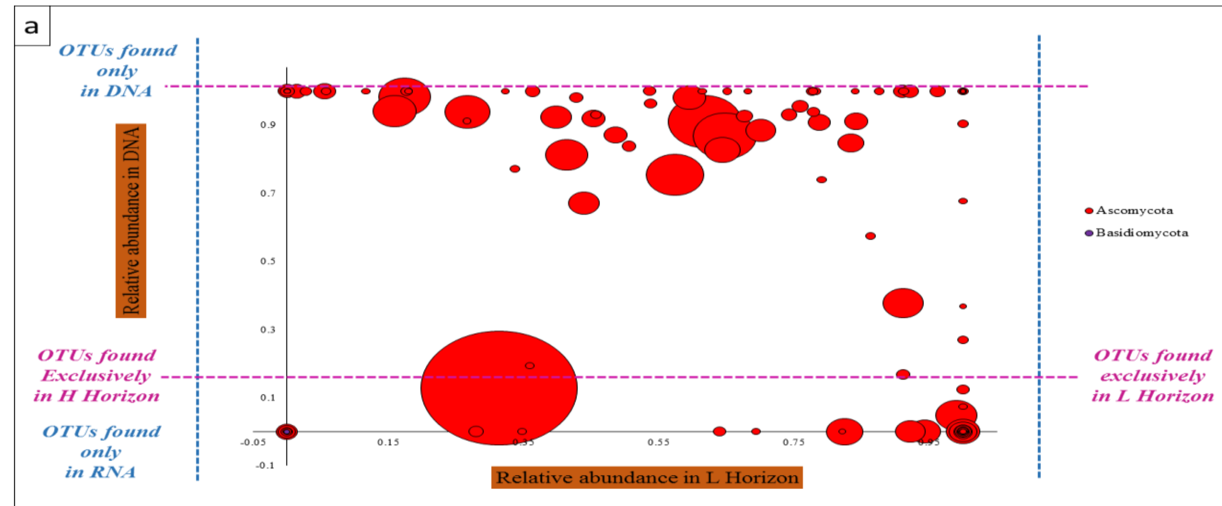
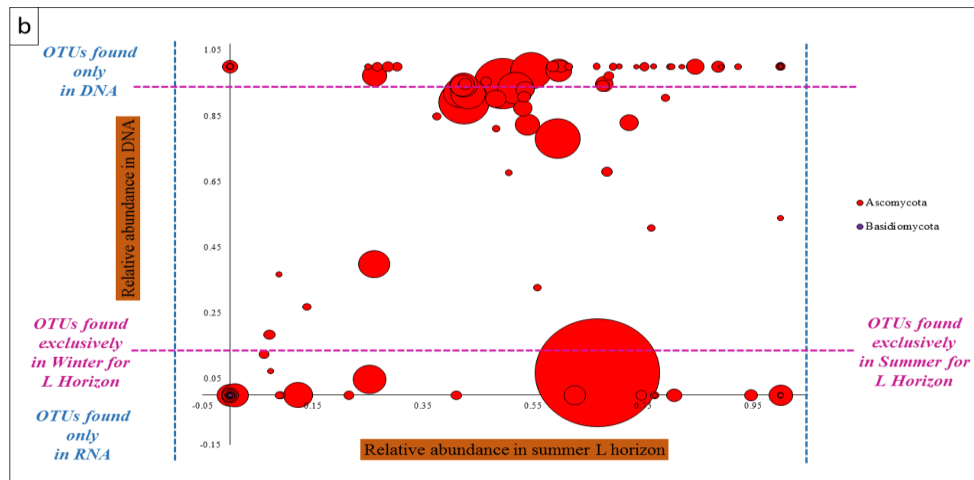


Figure 4. Distribution of major GH1 family fungal β-glucosidase gene OTUs from *Picea abies* forest litter and soil among horizons and seasons. a) between litter and H horizon and between DNA and RNA, b) between summer and winter and between DNA and RNA for litter horizon and c) between summer and winter and between DNA and RNA for soil.





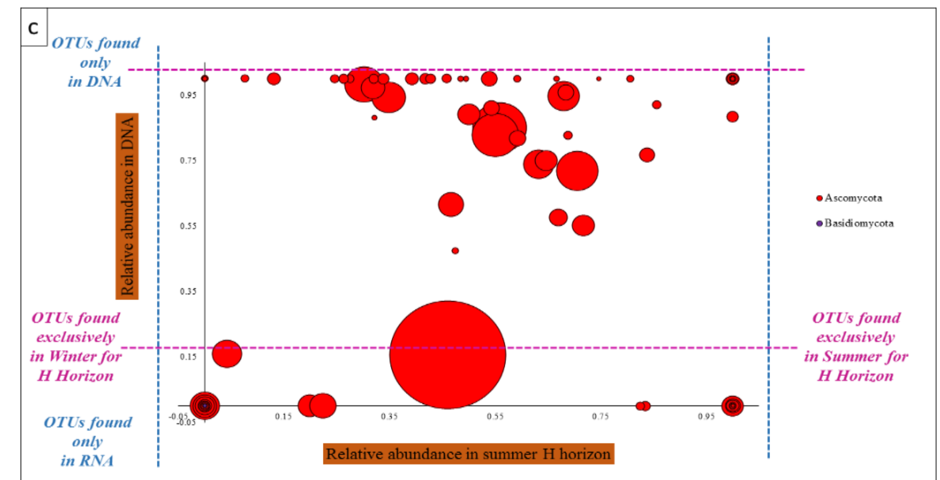
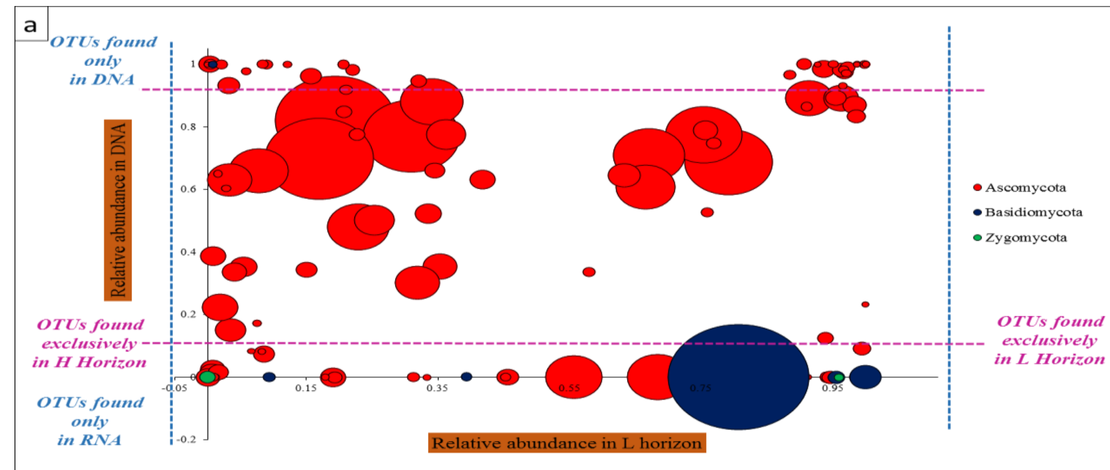
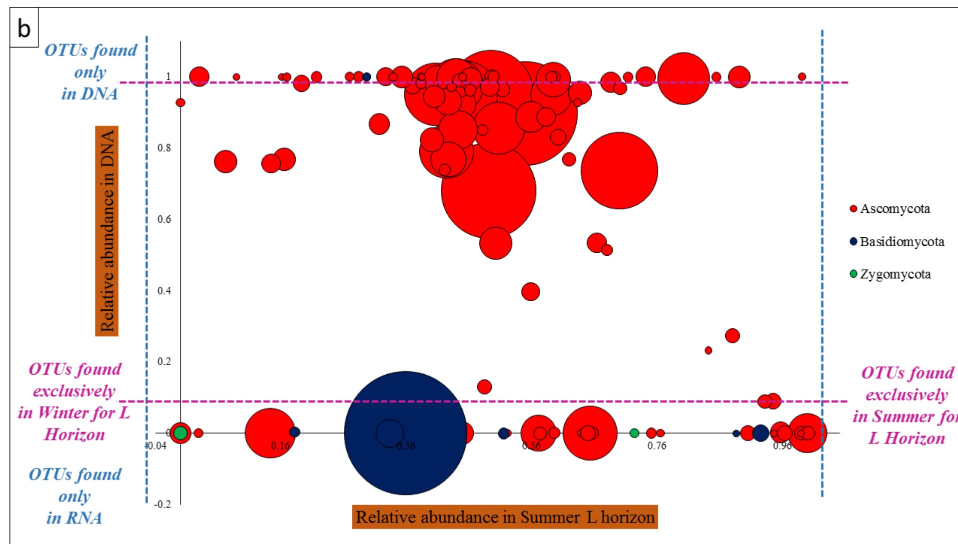


Figure 5. Distribution of major GH3 family fungal β-glucosidase gene OTUs from *Picea abies* forest litter and soil among horizons and seasons. a) between litter and H horizon and between DNA and RNA, b) between summer and winter and between DNA and RNA for litter horizon and c) between summer and winter and between DNA and RNA for soil.





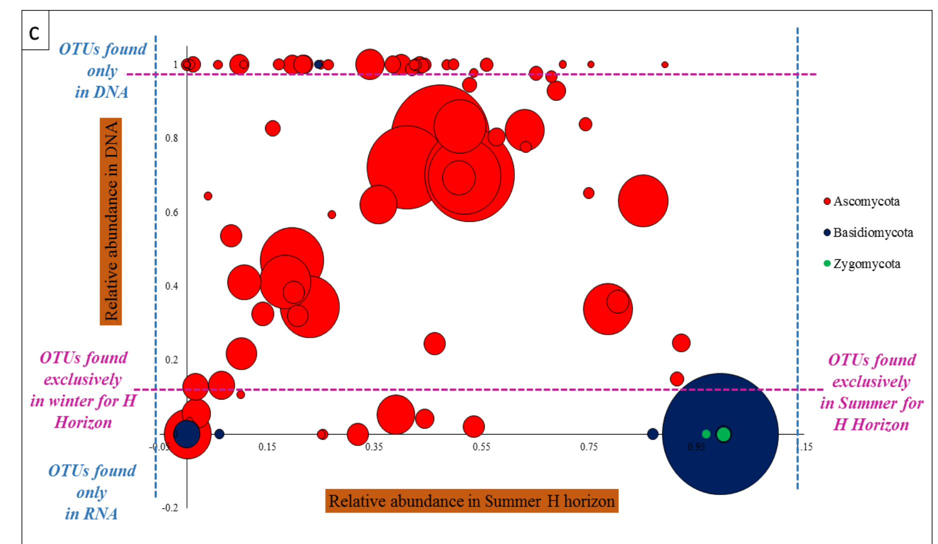


Table 1. List of primers used in this study.

β-glucosidase encoding gene Family/community	**Primer Name/Sequence (5'-3')**	**Amplicon Length (bp)**	**Reference**
GH1-Bacteria	β-gluF2: TTCYTBGGYRTCAACTACTA β-gluR4: CCGTTYTCGGTBAYSWAGA	180	Cañizares et al., 2011
GH3-Bacteria	BGH3BF: TTCGGCGAAGAYCC BGH3BR: ACGCCTTYRWARCC	200-300	Li et al., 2013
GH1-Fungi	bglFGH1F: TGGATCNTTCAAYGARCC bglFGH1R: GTAGTGGTTCAGCCRWARAA	350-500	This study
GH3-Fungi	bglFGH3F: GTTCCGTCATGTGCTCYTAYAA bglFGH3R: CATGATACGGGTAGCCATRTC	300	This study

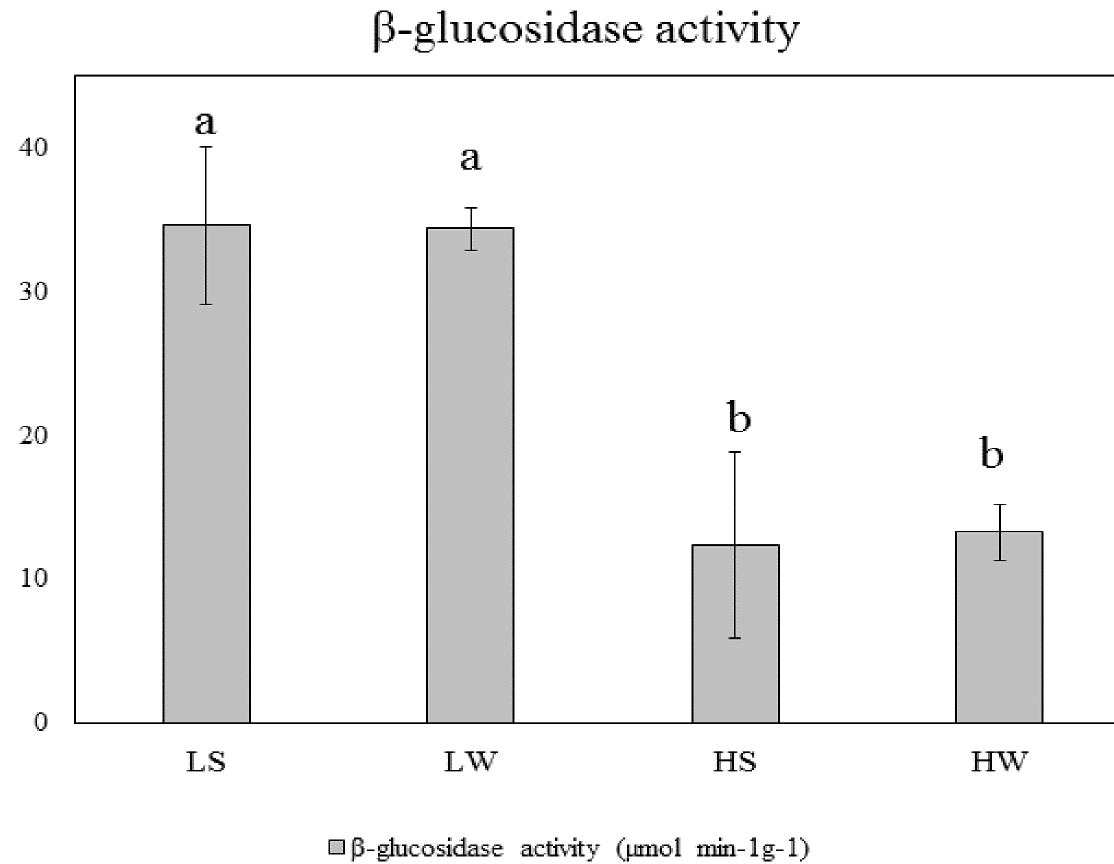
Table 2 Illumina MiSeq sequencing results obtained from this study.

	GH3_Bacteria	GH3_Fungi	GH1_Bacteria	GH1_Fungi
Total no. of sequences	1176416	634326	563763	11443
No. of singletons	40807	4533	7374	730
No. of OTUs with singletons	63325	7178	14698	1166
No. of OTUs without singletons	22518	2645	7324	436
No. of OTUs after removal of nonspecific sequences and singletons	2656	1165	3706	178
No. of sequences used in subsamples	2000	2000	2000	2000

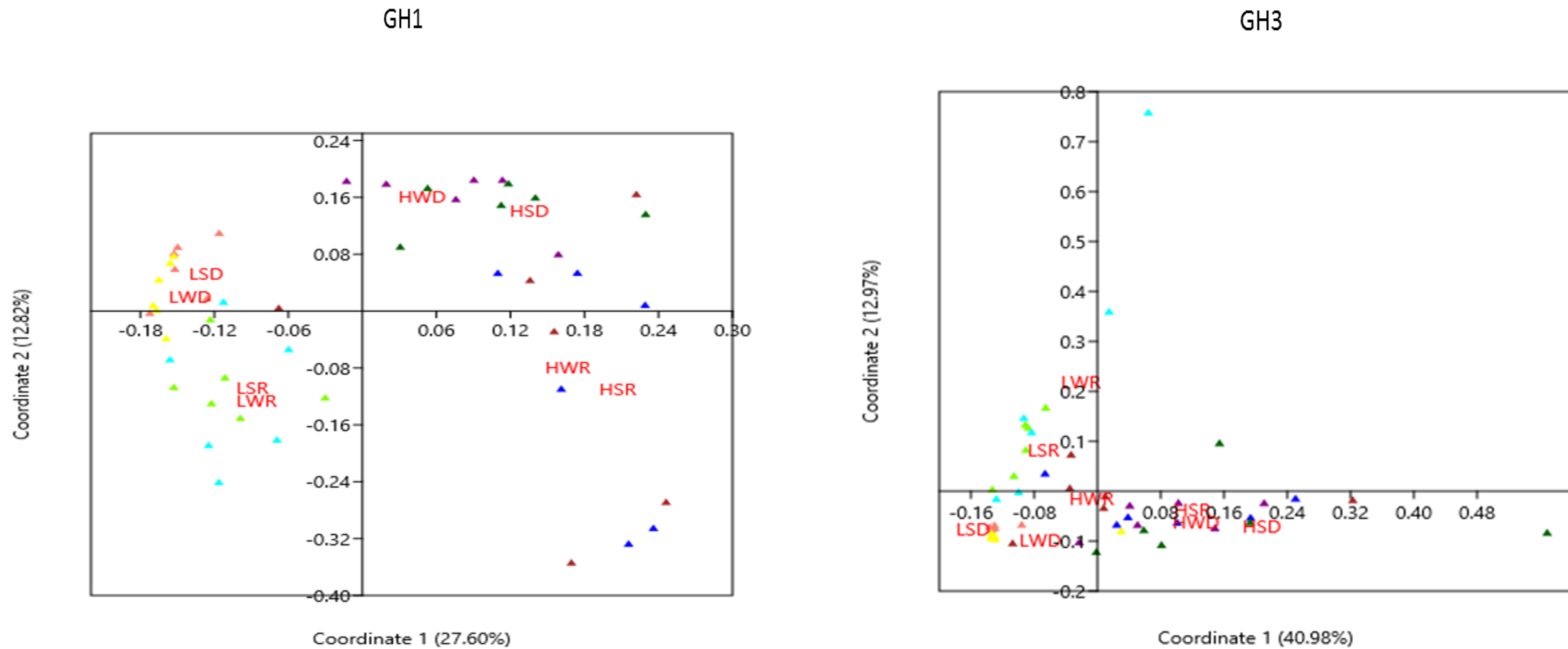
Table 3 Percentage of expressed β-glucosidase gene sequences in fungi and bacteria in litter and humic horizon during the winter and summer. The data represent means from 6 studied sites. Different letters indicate differences among mean values in columns. (One way ANOVA followed by Tukey post hoc test, $p < 0.05$).

	GH1_Bacteria	**GH3_Bacteria**	**GH1_Fungi**	**GH3_Fungi**
HS	57.53^{a}	39.46^{a}	24.17^{a}	32.58^{b}
HW	40.06^{a}	51.54^{b}	16.05^{a}	23.37^{b}
LS	62.87^{a}	29.91^{c}	23.42^{a}	69.72^{a}
LW	56.70^{a}	19.23^{c}	30.07^{a}	43.99^{a}

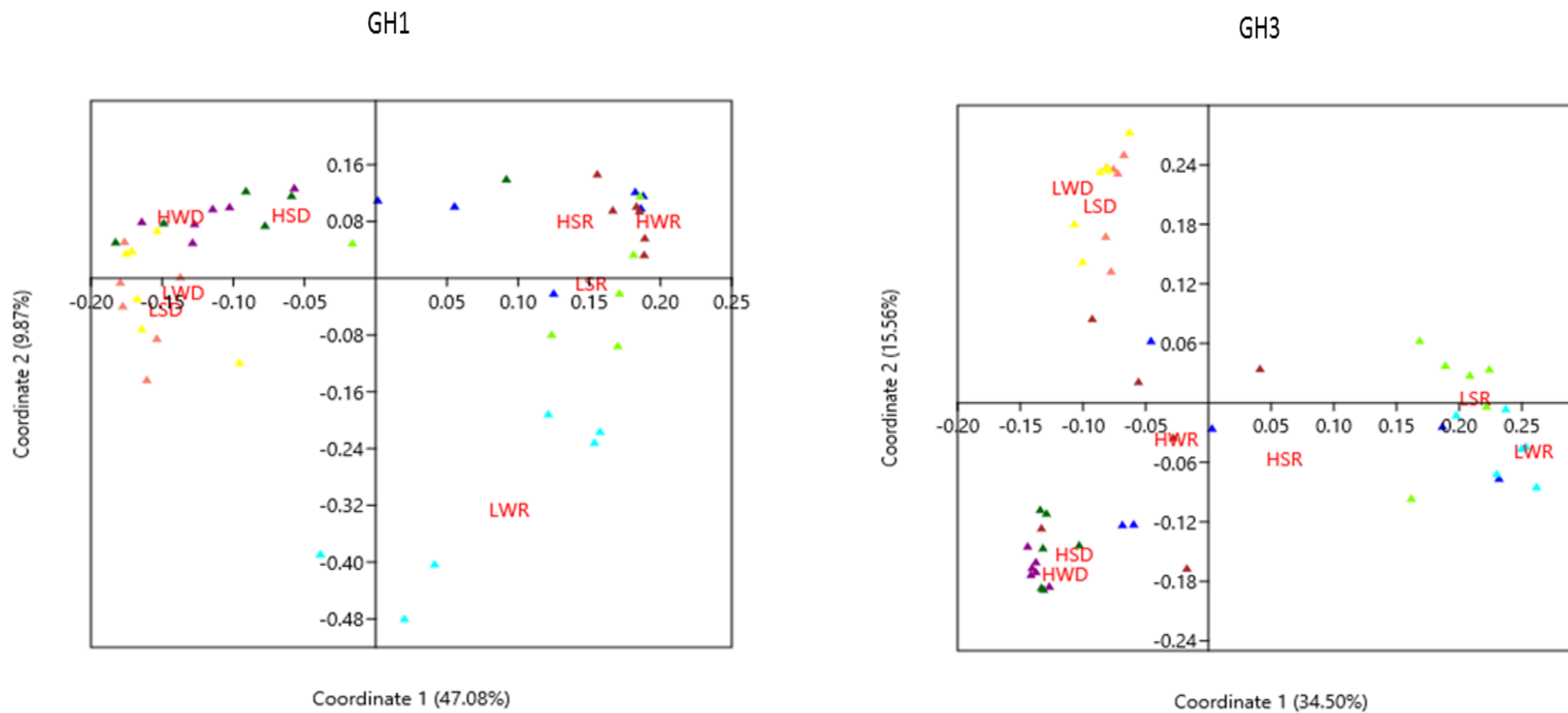
Supplementary Figure 1. β-Glucosidase activity in *Picea abies* forest litter and soil in summer and winter. The data represent the means of six replicates for each horizon and season. Significant differences are indicated by different letters ($P < 0.05$).



Supplementary Figure 2. Principal Coordinate Analysis (PCoA) based on Raup and Crick probability similarity index OTUs with abundance over > 0.3% of bacterial β-glucosidase genes from the *Picea abies* forest litter and soil in summer and winter.



Supplementary Figure 3. Principal Coordinate Analysis (PCoA) based on Raup and Crick probability similarity index OTUs with abundance over > 0.3% of fungal β-glucosidase genes from the *Picea abies* forest litter and soil in summer and winter.



Supplementary figure 4. Phylogenetic relationship among reference bacterial β-glucosidase encoded protein sequences and environmental sequences from this study. GH1 bacterial β-glucosidase sequences (a) and GH3 bacterial β-glucosidase sequences (b). Translated amino acid sequences of OTUs with abundance over > 0.3%. Sequences were aligned using MAFFT (Katoh et al., 2002) to all homologous sequences retrieved from CAZy database (http://www.cazy.org/). Maximum-Likelihood (ML) trees were generated by MEGA 6 (Tamura et al., 2013) using JTT model (Jones et al., 1992) for protein sequences. Robustness of the tree topology was tested by bootstrap analysis (100 replicates) and only bootstrap values ≥ 50 are shown. Representative actinobacteria, proteobacteria, firmicutes, acidobacteria and other low abundant phyla reference sequences are marked with dots in blue, pink, green, red and orange respectively.

Supplementary figure 5. Phylogenetic relationship among reference fungal β-glucosidase encoded protein sequences and environmental sequences from this study. GH1 fungal β-glucosidase sequences (a) and GH3 fungal β-glucosidase sequences (b). Translated amino acid sequences of OTUs with abundance over > 0.3%. Sequences were aligned using MAFFT (Katoh et al., 2002) to all homologous sequences retrieved from CAZy database (http://www.cazy.org/). Maximum-Likelihood (ML) trees were generated by MEGA 6 (Tamura et al., 2013) using JTT model (Jones et al., 1992) for protein sequences. Robustness of the tree topology was tested by bootstrap analysis (100 replicates) and only bootstrap values ≥ 50 are shown. Representative ascomycota, basidiomycota and zygomycota reference sequences are marked with dots in red, blue and green respectively.

a

b

Supplementary Table 1. Diversity of fungal and bacterial β-glucosidases from coniferous forest litter and soil belonging to the GH1 and GH3 families. The data represent means and standard deviations from six sites. Different letters indicate statistically significant differences among groups of samples (One-way ANOVA followed by Tukey post hoc test, $p < 0.05$).

a (GH1 bacterial β-glucosidase genes)	HSR	HWR	LSR	LWR	HSD	HWD	LSD	LWD
Shannon-wiener diversity index	2.57±0.83^{ab}	2.25±0.26^{b}	2.75±0.82^{ab}	2.78±0.53^{ab}	3.13±0.35^{ab}	3.24±0.36^{a}	2.87±0.31^{ab}	3.18±0.40^{ab}
OTU richness	20.83±16.13^{ab}	11.17±2.23^{b}	21.83±14.97^{ab}	21.17±12.17^{ab}	32.33±11.5^{ab}	34±10.33^{ab}	22.50±6.77^{ab}	36.67±12.75^{a}
Evenness	0.95±0.05^{a}	0.94±0.07^{a}	0.97±0.02^{a}	0.96±0.03^{a}	0.92±0.06^{a}	0.93±0.02^{a}	0.94±0.03^{a}	0.89±0.03^{a}
Chao-1	66.99±31.97^{abc}	31.97±21.99^{c}	51.25±38.61^{abc}	64.54±34.71^{bc}	119.97±47.34^{a}	133.9±122.9^{ab}	50.19±19.92^{abc}	111.62±45.66^{ab}

b (GH3 bacterial β-glucosidase genes)	HSR	HWR	LSR	LWR	HSD	HWD	LSD	LWD
Shannon-wiener diversity index	2.42±0.37^{bc}	2.6±0.5^{abc}	2.74±0.55^{abc}	2.04±0.29^{c}	2.8±0.53^{ab}	2.69±0.29^{abc}	3.18±0.26^{a}	2.95±0.22^{ab}
OTU richness	21.17±5.88^{a}	20.67±10.07^{a}	29.50±12.68^{a}	21.33±4.84^{a}	21.17±7.88^{a}	18.50±5.43^{a}	32.83±7.63^{a}	26.67±9.16^{a}
Evenness	0.8±0.06^{c}	0.88±0.04^{abc}	0.83±0.09^{bc}	0.67±0.08^{d}	0.95±0.04^{a}	0.94±0.04^{a}	0.92±0.05^{ab}	0.91±0.04^{ab}
Chao-1	59.74±14.27^{bc}	49.86±18.34^{c}	81.4±38.81^{abc}	71.49±32.79^{bc}	69.99±38.36^{bc}	104.5±68.79^{abc}	207.22±199.17^{a}	185.75±135.04^{ab}

c (GH1 fungi β-glucosidase genes)	HSR	HWR	LSR	LWR	HSD	HWD	LSD	LWD
Shannon-wiener diversity index	1.24±0.75^{b}	0.91±0.40^{b}	1.49±0.83^{b}	1.89±0.26^{b}	2.84±0.45^{b}	3.26±0.28^{a}	3.21±0.25^{a}	3.09±0.29^{a}
OTU richness	8±4.52^{c}	5.67±2.58^{c}	8.5±5.32^{c}	12.67±2.58^{c}	23±8.20^{b}	32.67±5.47^{a}	32.5±4.59^{a}	30.83±4.79^{ab}
Evenness	0.59±0.23^{b}	0.55±0.1^{b}	0.76±0.25^{ab}	0.75±0.09^{ab}	0.92±0.09^{a}	0.94±0.04^{a}	0.92±0.04^{a}	0.9±0.06^{a}
Chao-1	15.67±12.63^{bc}	10.08±7.53^{c}	17.09±16.71^{bc}	25.83±9.99^{bc}	53.14±23.46^{ab}	74.75±30.48^{a}	77.75±23.71^{a}	90.65±90.65^{a}

d (GH3 fungi β-glucosidase genes)	HSR	HWR	LSR	LWR	HSD	HWD	LSD	LWD
Shannon-wiener diversity index	1.67±0.58^{c}	2.01±0.25^{bc}	2.63±0.38^{ab}	1.88±0.66^{bc}	2.15±0.23^{abc}	2.3±0.25^{abc}	2.86±0.34^{a}	2.9±0.49^{a}
OTU richness	7.17±2.79^{d}	9.67±2.73^{d}	22.33±6.22^{abc}	14.33±5.54^{bcd}	12.17±2.14^{cd}	15.33±5.57^{bcd}	25±7.64^{ab}	26.67±9.85^{a}
Evenness	0.86±0.21^{ab}	0.9±0.04^{ab}	0.85±0.05^{ab}	0.71±0.17^{b}	0.87±0.08^{ab}	0.87±0.04^{ab}	0.9±0.03^{ab}	0.91±0.03^{a}
Chao-1	12.58±7.32^{b}	12.71±3.03^{b}	53.24±24^{a}	31.92±20.12^{ab}	23.58±4.13^{ab}	29.23±19.66^{ab}	51.95±24.92^{a}	52.56±24.78^{a}

Supplementary Table 2. Similarity of fungal and bacterial β-glucosidase gene and transcript pools from coniferous forest litter and soil belonging to the GH1 and GH3 families. Analysis of similarities (ANOSIM) of Bray-Curtis similarity measures (R): GH1 bacterial β-glucosidase genes (a), GH3 bacterial β-glucosidase genes (b), GH1 fungi β-glucosidase genes (c), GH3 fungal β-glucosidase genes (d) in coniferous forest soil The R values in ANOSIM ranges from 0 to 1, where R > 0.7 indicates significant difference, R < 0.25 high similarity and 0.7 < R > 0.25 moderate distribution. Significant dissimilarities (R > 0.7) indicated in bold script.

a (GH1 bacterial)	HSR	HWR	LSR	LWR	HSD	HWD	LSD
HWR	0.00						
LSR	**0.73**	0.50					
LWR	**0.70**	0.54	0.00				
HSD	0.23	0.19	0.50	**0.70**			
HWD	0.28	0.24	0.39	0.55	0.12		
LSD	**0.70**	0.48	0.12	0.20	**0.91**	**0.88**	
LWD	**0.71**	0.53	0.13	0.21	**0.96**	**0.91**	0.10

b (GH3 bacterial)	HSR	HWR	LSR	LWR	HSD	HWD	LSD
HWR	0.04						
LSR	0.64	0.27					
LWR	0.33	0.16	0.01				
HSD	0.02	0.00	0.44	0.25			
HWD	0.14	0.28	**0.80**	0.42	0.0		
LSD	**0.80**	0.53	0.62	0.38	0.57	**0.89**	
LWD	**0.70**	0.42	0.59	0.35	0.47	**0.70**	0.05

c (GH1 fungi)	HSR	HWR	LSR	LWR	HSD	HWD	LSD
HWR	0.07						
LSR	0.01	0.24					
LWR	0.41	0.55	0.22				
HSD	**0.76**	**0.89**	**0.76**	**0.86**			
HWD	**1.00**	**1.00**	**0.98**	**0.97**	0.03		
LSD	**0.92**	**1.00**	**0.94**	**0.92**	**0.70**	**0.96**	
LWD	**0.90**	**1.00**	**0.88**	**0.89**	0.38	**0.70**	0.00

d (GH3 fungi)	HSR	HWR	LSR	LWR	HSD	HWD	LSD
HWR	0.28						
LSR	0.24	**0.72**					
LWR	0.39	**0.73**	0.34				
HSD	0.52	0.21	**1.00**	**1.00**			
HWD	0.54	0.17	**1.00**	**1.00**	0.03		
LSD	0.57	0.29	**1.00**	**1.00**	**0.99**	**1.00**	
LWD	0.51	0.25	**1.00**	**1.00**	**0.99**	**1.00**	0.00

Supplementary Table 3: Overview of taxonomical assignment of most abundant OTUs of bacterial β-glucosidase genes from GH1 family, their abundance and districution among L and H horizons and among DNA and RNA in summer and winter. Abundance data are expressed as means (%) and standard deviations from six different sites. Statastically significant differences in relative abundance among HLR, HZR, LLR, LZR, HLD, HZD, LLD and LZD (ANOVA followed by TuKey Post-hoc) are indicated by different letters.

OTUs	Assignment	Mean Abundance (%)	HLR (%)	HZR (%)	LLR (%)	LZR (%)	HLD (%)	HZD (%)	LLD (%)	LZD (%)	Best Hit	E value	Similarity (%)	Coverage (%)
0	Actinobacteria	9.28	2.08±2.09 c	4.62±5.47 bc	11.76±6.75 ab	9.98±4.94 abc	6.94±2.18 abc	11.16±4.02 ab	13.83±2.29 a	13.84±2.22 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
1	Actinobacteria	7.66	0.37±0.45 d	1.15±2.21 d	10.80±5.11 abc	12.58±8.60 ab	2.92±1.33 cd	5.10±2.54 bcd	13.61±3.37 a	14.74±2.51 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
2	Actinobacteria	6.16	2.72±3.99 a	4.11±3.18 a	1.89±1.38 a	2.38±2.60 a	11.59±9.27 a	8.67±3.78 a	10.19±5.04 a	7.74±5.03 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
3	Actinobacteria	3.05	4.71±5.97 ab	2.6±5.64 ab	0.0±0.0 b	0.0±0.0 b	7.31±6.53 ab	9.20±4.22 a	0.24±0.29 b	0.32±0.60 b	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	1.12E-15	43.2	100
4	Actinobacteria	2.49	0.0±0.0 b	0.13±0.26 b	3.22±4.42 b	1.58±1.54 b	0.98±0.76 b	1.18±1.01 b	4.38±1.36 ab	8.43±3.8 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	4.70E-15	42	100
5	Actinobacteria	2.49	5.50±2.84 ab	7.1±8.13 a	0.37±0.46 b	0.09±0.12 b	3.38±1.49 ab	3.16±2.30 ab	0.21±0.19 b	0.12±0.07 b	ACZ90607.1|Beta-glucosidase [Streptosporangium roseum DSM 43021]	4.24E-25	62.1	100
6	Alphaproteobacteria	2.47	2.59±1.97 ab	5.67±6.55 ab	0.0±0.0 b	0.04±0.03 b	6.67±3.67 a	4.06±1.71 ab	0.58±0.49 b	0.13±0.08 b	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	5.20E-20	52.3	100
7	Actinobacteria	1.01	0.13±0.22 a	6.7±13.48 a	0.0±0.0 a	0.0±0.0 a	0.45±0.25 a	0.71±0.58 a	0.04±0.06 a	0.01±0.02 a	CBK71521.1|broad-specificity cellobiase [Bifidobacterium pseudocatenulatum D2CA]	7.98E-12	57.6	39.8
8	Deltaproteobacteria	1.09	1.38±1.49 a	4.94±9.72 a	0.57±0.64 a	0.07±0.08 a	0.95±0.39 a	0.46±0.31 a	0.25±0.21 a	0.08±0.05 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	4.38E-30	67.2	100
9	Actinobacteria	1.87	0.34±0.50 a	0.74±1.04 a	2.89±3.44 a	3.16±1.96 a	0.97±0.47 a	1.32±0.47 a	3.11±1.3 a	2.45±0.82 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
10	Actinobacteria	1.46	0.0±0.0 b	0.11±0.24 ab	2.99±4.31 ab	0.40±0.63 ab	1.06±1.53 ab	0.41±0.39 ab	3.29±0.61 a	3.44±0.66 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
11	Actinobacteria	1.40	0.82±1.21 a	2.48±4.15 a	0.16±0.34 a	0.08±0.18 a	2.70±2.44 a	4.87±7.09 a	0.04±0.05 a	0.02±0.01 a	CAA82733.1|Beta-glucosidase [Streptomyces sp.]	1.26E-14	40.7	100
12	Actinobacteria	1.77	0.56±0.57 a	1.43±2.17 a	2.86±2.15 a	2.33±1.65 a	1.35±0.50 a	1.63±0.8 a	2.14±0.68 a	1.88±0.47 a	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	1.78E-13	46.4	69.1
13	Actinobacteria	1.43	0.0±0.0 a	0.39±0.86 a	1.5±2.42 a	2.71±2.61 a	0.81±1.07 a	0.66±0.33 a	2.93±1.51 a	2.46±1.76 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	3.84E-14	48.2	69.1
14	Actinobacteria	1.56	0.09±0.14 c	0.83±1.78 bc	1.85±0.71 abc	3.61±2.16 a	0.53±0.23 bc	0.97±0.68 bc	2.19±0.41 abc	2.45±1.03 ab	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	1.74E-14	48.2	69.1
15	Actinobacteria	1.03	0.33±0.75 b	0.26±0.58 b	1.11±2.49 ab	0.21±0.46 b	2.02±1.03 ab	0.62±0.71 b	3.17±1.43 a	0.51±0.17 b	CAM03371.1|beta-glucosidase [Saccharopolyspora erythraea NRRL 2338]	3.04E-08	47.1	43
16	Actinobacteria	0.95	0.32±0.49 bc	0.85±0.81 bc	0.16±0.27 c	0.50±0.44 bc	1.44±0.62 ab	2.32±0.99 a	1.12±0.68 abc	0.91±0.29 bc	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	8.20E-14	48.2	69.1
17	Actinobacteria	1.08	0.68±0.86 a	1.3±1.33 a	0.63±0.81 a	0.75±1.21 a	1.88±1.42 a	1.14±0.69 a	1.4±0.93 a	0.83±0.34 a	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	1.74E-14	48.2	69.1
18	Actinobacteria	1.00	0.62±1.35 a	0.0±0.0 a	1.75±2.57 a	1.5±2.12 a	0.47±0.30 a	0.12±0.04 a	1.98±0.77 a	1.55±0.99 a	AIR98549.1|Bifunctional beta-D-glucosidase/beta-D-fucosidase [Streptomyces glaucescens]	2.86E-12	64.5	40.8
19	Betaproteobacteria	0.75	0.26±0.32 a	0.01±0.01 a	0.48±0.8 a	1.55±2.26 a	0.65±0.56 a	0.91±1.03 a	0.95±0.43 a	1.19±0.24 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	8.75E-35	85.7	100
20	Gammaproteobacteria	0.79	1.36±1.9 a	0.18±0.26 a	1.02±2.01 a	0.15±0.32 a	0.92±0.79 a	1.28±0.82 a	0.88±1.37 a	0.54±0.69 a	AFC17959.1|beta-glucosidase, partial [Pseudomonas sp. RBE6]	1.06E-12	36.9	100
21	Deinococcus-Thermus	0.34	2.71±6.04 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AIZ45048.1|Beta-glucosidase [Deinococcus swuensis]	1.09E-13	60.5	59.4
23	Alphaproteobacteria	0.51	0.32±0.46 bc	0.01±0.2 c	0.0±0.0 c	0.01±0.02 c	2.07±1.19 a	1.52±1.14 ab	0.05±0.05 c	0.05±0.07 c	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	5.20E-20	52.3	100
24	Alphaproteobacteria	0.74	0.85±0.52 ab	1.62±1.47 a	0.03±0.07 b	0.01±0.02 b	2.15±1.27 a	1.06±0.34 ab	0.13±0.09 b	0.03±0.02 b	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	2.54E-19	49.2	100
26	Actinobacteria	0.56	0.0±0.0 b	0.01±0.01 b	0.01±0.01 b	1.25±1.78 ab	0.16±0.31 b	0.05±0.04 b	1.25±0.29 ab	1.75±1.01 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	2.48E-14	48.2	69.1
27	Alphaproteobacteria	0.55	0.61±0.37 abc	1.36±1.37 ab	0.0±0.0 c	0.02±0.02 c	1.53±0.95 a	0.77±0.34 abc	0.12±0.12 bc	0.02±0.01 c	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	1.71E-18	51.6	98.5
29	Actinobacteria	0.90	0.0±0.0 a	0.0±0.0 a	1.16±2.52 a	4.41±8.14 a	0.19±0.38 a	0.1±0.16 a	0.68±0.41 a	0.68±0.28 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	2.48E-14	48.2	69.1
30	Actinobacteria	0.94	0.6±1.3 a	0.7±1.55 a	0.91±1.51 a	2.85±4.09 a	0.09±0.11 a	0.03±0.03 a	0.93±0.95 a	1.41±1.51 a	AJE44123.1|beta-glucosidase [Streptomyces nodosus]	3.50E-13	45.8	59.3
31	Actinobacteria	0.57	1.31±1.71 a	0.56±1.24 a	0.0±0.0 a	0.0±0.0 a	1.39±1.23 a	1.21±0.67 a	0.03±0.03 a	0.03±0.05 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	1.34E-15	40.7	100
37	Actinobacteria	0.64	0.10±0.17 a	0.18±0.33 a	2.91±4.54 a	0.22±0.42 a	0.08±0.1 a	0.08±0.05 a	0.55±0.27 a	0.95±0.32 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	6.17E-15	42	100
38	Actinobacteria	0.63	1.11±1.11 a	2.38±3.72 a	0.09±0.19 a	0.04±0.06 a	0.80±0.57 a	0.57±0.44 a	0.03±0.02 a	0.02±0.02 a	ACZ90607.1|Beta-glucosidase [Streptosporangium roseum DSM 43021]	3.09E-25	63.6	100
39	Deinococcus-Thermus	0.91	2.79±4.25 a	0.82±0.9 a	0.93±1.52 a	2.76±2.55 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ABF44291.1|broad-specificity cellobiase [Deinococcus geothermalis DSM 11300]	5.74E-19	80.6	59
40	Deltaproteobacteria	0.32	0.93±1.01 a	0.07±0.13 a	0.0±0.0 a	0.0±0.0 a	0.34±0.22 a	1.20±1.7 a	0.01±0.01 a	0.01±0.01 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	4.69E-31	70.1	100
41	Alphaproteobacteria	0.32	1.26±2.82 a	1.22±2.6 a	0.0±0.0 a	0.0±0.0 a	0.05±0.01 a	0.05±0.07 a	0.0±0.0 a	0.0±0.0 a	CCD00112.1|Beta-glucosidase A [Azospirillum brasilense Sp245]	1.04E-22	52.3	100
42	Actinobacteria	0.32	0.0±0.0 b	0.0±0.0 b	0.14±0.15 b	0.01±0.01 b	0.09±0.18 b	0.01±0.01 b	0.97±0.65 ab	1.36±1.43 a	AFR27030.1|Beta-glucosidase A [Arthrobacter sp. Rue61a]	3.09E-34	66.7	100
43	Actinobacteria	0.39	0.0±0.0 b	0.05±0.12 b	0.64±0.76 ab	0.38±0.43 b	0.2±0.15 b	0.15±0.14 b	0.61±0.17 ab	1.12±0.4 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	2.99E-15	42	100
44	Actinobacteria	0.41	0.0±0.0 a	0.05±0.1 a	0.25±0.54 a	1.05±1.46 a	0.16±0.2 a	0.08±0.04 a	0.80±0.24 a	0.93±0.34 a	AJE44123.1|beta-glucosidase [Streptomyces nodosus]	1.34E-12	45.8	59.3
45	Bacteroidetes	0.43	0.36±0.76 a	1.2±1.86 a	0.08±0.19 a	0.02±0.04 a	0.19±0.2 a	0.45±0.69 a	0.41±0.2 a	0.76±0.58 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	9.39E-16	71.4	53.8
46	Unknown	0.41	0.18±0.44 a	0.01±0.01 a	1.87±4.12 a	0.30±0.66 a	0.16±0.11 a	0.39±0.38 a	0.1±0.11 a	0.10±0.07 a	AFC17950.1|putative beta-glucosidase, partial [uncultured bacterium]	7.14E-12	35.4	100
47	Actinobacteria	0.32	0.92±0.38 a	0.81±0.84 a	0.05±0.07 b	0.01±0.03 b	0.45±0.17 ab	0.3±0.22 ab	0.03±0.01 b	0.01±0.02 b	ACZ90607.1|Beta-glucosidase [Streptosporangium roseum DSM 43021]	1.51E-24	64.6	98.5
50	Thermotogae	0.52	0.58±0.9 a	1.78±3.17 a	0.53±1.10 a	0.12±0.23 a	0.29±0.24 a	0.16±0.12 a	0.42±0.36 a	0.27±0.24 a	ABR31482.1|Beta-glucosidase [Thermosipho melanesiensis BI429]	3.42E-06	43.8	47.1
51	Bacteroidetes	0.25	0.01±0.02 b	0.29±0.41 ab	0.0±0.0 b	0.01±0.02 b	0.17±0.07 ab	0.52±0.32 a	0.38±0.19 ab	0.61±0.36 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	8.77E-18	71.4	53.8
52	Unknown	0.31	0.53±0.58 a	0.1±0.19 a	0.21±0.35 a	0.07±0.09 a	0.42±0.44 a	0.48±0.27 a	0.27±0.49 a	0.37±0.67 a	AFC17952.1|putative beta-glucosidase, partial [uncultured bacterium]	3.78E-12	38.5	100
53	Actinobacteria	0.38	0.29±0.64 a	0.0±0.0 a	0.78±1.49 a	0.72±1.17 a	0.13±0.1 a	0.03±0.02 a	0.58±0.21 a	0.51±0.42 a	AIR98549.1|Bifunctional beta-D-glucosidase/beta-D-fucosidase [Streptomyces glaucescens]	8.52E-12	58.1	40.8
54	Gammaproteobacteria	0.32	0.24±0.25 a	0.05±0.07 a	0.95±2.11 a	0.28±0.62 a	0.21±0.28 a	0.58±0.64 a	0.11±0.14 a	0.14±0.17 a	AFC17959.1|beta-glucosidase, partial [Pseudomonas sp. RBE6]	1.46E-12	35.4	100
55	Alphaproteobacteria	0.34	0.42±0.53 a	0.93±1.26 a	0.06±0.14 a	0.02±0.04 a	0.86±0.63 a	0.34±0.14 a	0.06±0.04 a	0.01±0.01 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	5.20E-20	52.3	100
56	Deltaproteobacteria	0.19	0.3±0.34 a	0.78±1.51 a	0.12±0.14 a	0.01±0.01 a	0.18±0.06 a	0.08±0.07 a	0.06±0.04 a	0.01±0.01 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	5.38E-29	65.7	100
58	Actinobacteria	0.22	0.03±0.06 b	0.0±0.0 b	0.09±0.22 b	0.0±0.0 b	0.33±0.14 b	0.84±0.46 a	0.32±0.25 b	0.18±0.07 b	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	3.23E-14	48.2	69.1
59	Actinobacteria	0.25	0.16±0.16 ab	0.13±0.28 ab	0.25±0.55 ab	0.05±0.11 b	0.45±0.22 ab	0.12±0.12 ab	0.71±0.39 a	0.12±0.05 ab	CCE74801.1|beta-glucosidase [Clavibacter michiganensis subsp. nebraskensis NCPPB 2581]	1.34E-06	56.7	38
60	Actinobacteria	0.19	0.0±0.0 a	0.0±0.0 a	0.07±0.15 a	0.24±0.52 a	0.47±0.54 a	0.14±0.25 a	0.19±0.22 a	0.42±0.73 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
61	Actinobacteria	0.20	0.0±0.0 b	0.0±0.0 b	0.27±0.61 ab	0.03±0.05 b	0.24±0.49 ab	0.03±0.03 b	0.32±0.11 ab	0.72±0.49 a	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	2.84E-13	50	59.3
64	Actinobacteria	0.28	0.34±0.49 a	0.57±1.21 a	0.05±0.1 a	0.18±0.39 a	0.26±0.11 a	0.38±0.17 a	0.26±0.12 a	0.20±0.11 a	AIR98549.1|Bifunctional beta-D-glucosidase/beta-D-fucosidase [Streptomyces glaucescens]	7.29E-15	68.6	45.5
66	Actinobacteria	0.23	0.0±0.0 b	0.27±0.59 b	0.0±0.0 b	0.0±0.0 b	0.20±0.12 b	0.18±0.18 b	0.79±0.18 a	0.42±0.1 ab	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
68	Thermotogae	0.11	0.87±1.94 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ABR31482.1|Beta-glucosidase [Thermosipho melanesiensis BI429]	1.31E-08	55.6	42.9
69	Actinobacteria	0.11	0.86±1.92 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEI10439.1|putative beta-glucosidase [Corynebacterium resistens DSM 45100]	3.72E-12	48.2	100
70	Firmicutes	0.12	0.0±0.0 a	1.0±2.16 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEW05616.1|broad-specificity cellobiase [Sulfobacillus acidophilus DSM 10332]	9.42E-10	51.2	57.3
71	Actinobacteria	0.17	0.03±0.08 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.41±0.29 ab	0.9±1.0 a	0.02±0.0 b	0.0±0.0 b	CAJ89567.1|putative beta-glucosidase [Streptomyces ambofaciens ATCC 23877]	2.82E-15	40.7	100
72	Actinobacteria	0.15	0.05±0.08 a	0.95±1.66 a	0.0±0.0 a	0.0±0.0 a	0.08±0.04 a	0.11±0.09 a	0.0±0.0 a	0.0±0.0 a	CBK71521.1|broad-specificity cellobiase [Bifidobacterium pseudocatenulatum D2CA]	1.68E-12	60.6	39.8
73	Actinobacteria	0.21	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.54±0.88 a	0.08±0.06 a	0.09±0.08 a	0.42±0.15 a	0.54±0.32 a	AJE44123.1|beta-glucosidase [Streptomyces nodosus]	1.34E-12	45.8	59.3
74	Actinobacteria	0.25	0.25±0.34 a	0.49±0.69 a	0.09±0.21 a	0.05±0.11 a	0.49±0.44 a	0.61±0.97 a	0.01±0.01 a	0.0±0.0 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	1.63E-14	39.5	100
75	Bacteroidetes	0.28	0.13±0.18 a	0.85±1.82 a	0.01±0.02 a	0.02±0.04 a	0.12±0.14 a	0.31±0.43 a	0.20±0.11 a	0.58±0.50 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	9.39E-16	71.4	53.8
76	Actinobacteria	0.17	0.2±0.42 abc	0.13±0.22 bc	0.0±0.0 c	0.0±0.0 c	0.59±0.24 a	0.41±0.13 ab	0.03±0.02 bc	0.01±0.01 c	ABP54026.1|beta-glucosidase glycosyl hydrolase family 1 [Salinispora tropica CNB-440]	1.10E-09	65.5	34.9
78	Betaproteobacteria	0.28	0.0±0.0 a	0.04±0.08 a	1.35±2.68 a	0.30±0.35 a	0.01±0.01 a	0.0±0.0 a	0.29±0.16 a	0.26±0.13 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	4.64E-35	87.3	100
79	Alphaproteobacteria	0.30	0.17±0.36 a	0.01±0.02 a	2.15±4.78 a	0.09±0.21 a	0.0±0.0 a	0±0 a	0.0±0.0 a	0.0±0.0 a	ADO42989.1|Beta-glucosidase [Ketogulonicigenium vulgare Y25]	4.10E-06	59.3	33.3
80	Alphaproteobacteria	0.34	0.0±0.0 a	0.0±0.0 a	1.63±3.66 a	0.0±0.0 a	0.09±0.04 a	0.13±0.19 a	0.54±0.61 a	0.34±0.64 a	BAI76508.1|beta-glucosidase [Azospirillum sp. B510]	8.19E-25	60	100
81	Actinobacteria	0.30	0.0±0.0 a	0.42±0.92 a	0.41±0.81 a	0.39±0.65 a	0.07±0.1 a	0.03±0.04 a	0.53±0.24 a	0.52±0.13 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
82	Actinobacteria	0.19	0.34±0.76 a	0.41±0.82 a	0.0±0.0 a	0.0±0.0 a	0.04±0.04 a	0.74±1.3 a	0.01±0.01 a	0.0±0.0 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	4.49E-15	42	100
84	Actinobacteria	0.21	0.09±0.12 a	0.0±0.0 a	0.16±0.29 a	0.52±0.77 a	0.18±0.14 a	0.21±0.27 a	0.24±0.12 a	0.30±0.14 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	8.20E-14	48.2	69.1
83	Betaproteobacteria	0.15	0.0±0.0 c	0.02±0.02 c	0.0±0.0 c	0.0±0.0 c	0.33±0.13 ab	0.54±0.30 a	0.17±0.12 bc	0.15±0.19 bc	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	3.12E-34	84.1	100
87	Gammaproteobacteria	0.23	0.0±0.0 a	0.0±0.0 a	0.07±0.17 a	1.38±1.98 a	0.0±0.0 a	0.0±0.0 a	0.09±0.08 a	0.27±0.46 a	CAA56282.1|beta-glucosidase [Pantoea agglomerans]	1.71E-18	55.4	100
88	Actinobacteria	0.15	0.03±0.07 b	0.11±0.19 b	0.0±0.0 b	0.04±0.06 b	0.07±0.02 b	0.35±0.28 ab	0.06±0.05 b	0.5±0.40 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	3.87E-13	44.6	69.1
90	Actinobacteria	0.52	0.63±1.38 a	3.29±6.98 a	0.09±0.21 a	0.12±0.27 a	0.02±0.03 a	0.03±0.06 a	0.0±0.0 a	0.0±0.0 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	3.87E-13	44.6	69.1
91	Alphaproteobacteria	0.20	0.0±0.0 a	0.09±0.2 a	0.27±0.4 a	0.42±0.33 a	0.11±0.15 a	0.08±0.05 a	0.32±0.16 a	0.33±0.31 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	1.08E-23	60	100
94	Actinobacteria	0.33	1±1.21 a	0.34±0.49 a	0.27±0.38 a	1.04±0.81 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	1.01E-13	48.2	69.1
95	Deinococcus-Thermus	0.35	0.0±0.0 a	0.0±0.0 a	0.81±1.77 a	1.73±3.52 a	0.03±0.6 a	0.02±0.04 a	0.12±0.07 a	0.12±0.1 a	ABF44291.1|broad-specificity cellobiase [Deinococcus geothermalis DSM 11300]	7.28E-19	80.6	59
98	Actinobacteria	0.24	0.0±0.0 a	0.26±0.55 a	0.34±0.67 a	0.29±0.42 a	0.08±0.1 a	0.03±0.03 a	0.60±0.32 a	0.33±0.13 a	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	1.52E-13	48.2	69.1
99	Actinobacteria	0.12	0.05±0.1 b	0.04±0.08 b	0.0±0.0 b	0.0±0.0 b	0.33±0.18 ab	0.54±0.52 a	0.0±0.0 b	0.01±0.01 b	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	8.20E-14	48.2	69.1
100	Alphaproteobacteria	0.11	0.09±0.12 b	0.01±0.01 b	0.0±0.0 b	0.0±0.0 b	0.5±0.3 a	0.27±0.19 ab	0.01±0.02 b	0.01±0.03 b	CAA82733.1|Beta-glucosidase [Streptomyces sp.]	1.26E-14	40.7	100
103	Alphaproteobacteria	0.12	0.09±0.15 bc	0.05±0.08 c	0.0±0.0 c	0.0±0.0 c	0.5±0.19 a	0.27±0.16 b	0.02±0.01 c	0.02±0.04 c	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	2.54E-19	49.2	100
105	Actinobacteria	0.16	0.09±0.13 ab	0.18±0.15 ab	0.04±0.07 b	0.11±0.16 ab	0.24±0.1 ab	0.31±0.14 a	0.17±0.01 ab	0.12±0.04 ab	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	1.52E-13	48.2	69.1
106	Actinobacteria	0.09	0.02±0.03 a	0.67±1.39 a	0.0±0.0 a	0.0±0.0 a	0.03±0.02 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	CBK71521.1|broad-specificity cellobiase [Bifidobacterium pseudocatenulatum D2CA]	3.55E-08	60.9	28.4
108	Actinobacteria	0.20	0.13±0.23 a	0.11±0.24 a	0.7±1.04 a	0.12±0.14 a	0.02±0.03 a	0.03±0.02 a	0.17±0.09 a	0.34±0.1 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	5.14E-15	40.7	100
110	Alphaproteobacteria	0.22	0.86±1.91 a	0.13±0.28 a	0.0±0.0 a	0.01±0.02 a	0.46±0.21 a	0.23±0.18 a	0.03±0.01 a	0.01±0.01 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	2.00E-20	53.8	100
111	Actinobacteria	0.16	0.0±0.0 a	0.0±0.0 a	0.53±0.98 a	0.0±0.0 a	0.11±0.2 a	0.05±0.05 a	0.29±0.15 a	0.3±0.08 a	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	2.37E-14	48.2	69.1
112	Gammaproteobacteria	0.23	0.62±1.38 a	1.12±2.13 a	0.0±0.0 a	0.0±0.0 a	0.02±0.01 a	0.11±0.14 a	0.0±0.0 a	0.0±0.0 a	AAZ50390.1|beta-glucosidase, partial [Shewanella sp. G5]	1.98E-18	63.4	70.7
113	Deinococcus-Thermus	0.07	0.53±1.18 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AIZ45048.1|Beta-glucosidase [Deinococcus swuensis]	1.48E-13	57.9	59.4
118	Actinobacteria	0.12	0.0±0.02 b	0.01±0.02 b	0.0±0.0 b	0.18±0.29 ab	0.05±0.09 b	0.02±0.01 b	0.31±0.16 ab	0.40±0.29 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	2.48E-14	48.2	69.1
120	Betaproteobacteria	0.13	0.0±0.0 a	0.46±0.96 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.17±0.27 a	0.39±0.76 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	5.38E-34	84.1	100
121	Deltaproteobacteria	0.09	0.12±0.17 a	0.53±0.88 a	0.02±0.03 a	0.0±0.0 a	0.05±0.03 a	0.02±0.03 a	0.0±0.0 a	0.0±0.0 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	4.38E-29	67.2	100
122	Betaproteobacteria	0.23	0.0±0.0 a	0.02±0.04 a	0.34±0.39 a	1.35±2.69 a	0.01±0.02 a	0.0±0.0 a	0.07±0.1 a	0.08±0.08 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	5.88E-34	87.3	100
124	Actinobacteria	0.12	0.27±0.39 a	0.08±0.16 a	0.0±0.0 a	0.0±0.0 a	0.27±0.14 a	0.33±0.11 a	0.01±0.01 a	0.0±0.0 a	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	1.27E-14	44.6	69.1
127	Nitrospirae	0.06	0.44±0.98 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ACI21065.1|chain A, Beta-Glycosidase From Pyrococcus Horikoshii [Thermodesulfovibrio yellowstonii DSM	7.12E-06	57.7	41.9
128	Betaproteobacteria	0.13	0.03±0.05 a	0.01±0.02 a	0.08±0.15 a	0.36±0.59 a	0.13±0.11 a	0.11±0.11 a	0.14±0.07 a	0.17±0.05 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	5.43E-33	82.3	98.4
129	Deltaproteobacteria	0.14	0.0±0.0 a	0.0±0.0 a	0.65±1.46 a	0.0±0.0 a	0.02±0.03 a	0.01±0.01 a	0.29±0.48 a	0.11±0.09 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	1.33E-30	70.1	100
130	Gammaproteobacteria	0.14	0.0±0.0 a	0.0±0.0 a	0.13±0.23 a	0.75±1.14 a	0.0±0.0 a	0.0±0.0 a	0.10±0.06 a	0.14±0.1 a	CAA56282.1|beta-glucosidase [Pantoea agglomerans]	4.43E-18	53.8	100
132	Actinobacteria	0.11	0.0±0.0 b	0.01±0.01 b	0.0±0.0 b	0.0±0.0 b	0.08±0.03 ab	0.1±0.1 ab	0.42±0.46 a	0.29±0.25 ab	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.79E-14	46.4	69.1
131	Alphaproteobacteria	0.29	0.42±0.93 a	0.4±0.78 a	0.0±0.0 a	1.09±2.15 a	0.15±0.08 a	0.22±0.29 a	0.0±0.0 a	0.0±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	4.43E-22	56.9	100
135	Deltaproteobacteria	0.11	0.84±1.56 a	0.02±0.05 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	ADO74619.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	3.88E-09	54.3	50.7
136	Bacteroidetes	0.14	0.21±0.46 a	0.46±0.74 a	0.04±0.08 a	0.0±0.0 a	0.07±0.07 a	0.12±0.19 a	0.10±0.07 a	0.13±0.09 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	1.28E-15	68.6	53.8
138	Alphaproteobacteria	0.15	0.14±0.31 a	0.64±1.39 a	0.0±0.0 a	0.0±0.0 a	0.26±0.18 a	0.18±0.17 a	0.0±0.0 a	0.01±0.02 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	2.00E-16	50.8	100
141	Actinobacteria	0.09	0.0±0.0 c	0.0±0.0 c	0.02±0.06 bc	0.01±0.02 c	0.03±0.02 bc	0.04±0.05 bc	0.37±0.28 a	0.27±0.22 ab	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	4.49E-15	42	100
142	Actinobacteria	0.05	0.4±0.89 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AGT83082.1|beta-glucosidase [Amycolatopsis mediterranei RB]	1.40E-13	65	56.3
148	Deinococcus-Thermus	0.22	0.63±0.8 a	0.21±0.24 a	0.21±0.32 a	0.68±0.59 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ABF44291.1|broad-specificity cellobiase [Deinococcus geothermalis DSM 11300]	1.75E-19	80.6	59
149	Actinobacteria	0.12	0.18±0.27 b	0.08±0.16 b	0.0±0.0 b	0.0±0.0 b	0.62±0.41 a	0.04±0.03 b	0.0±0.0 b	0.0±0.0 b	AGF63732.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	6.35E-05	43.2	48.1
152	Deltaproteobacteria	0.09	0.38±0.5 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	0.01±0.06 a	0.21±0.32 a	0.0±0.0 a	0.0±0.0 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	2.52E-30	68.7	100
153	Actinobacteria	0.10	0.33±0.74 a	0.02±0.04 a	0.0±0.0 a	0.0±0.0 a	0.17±0.19 a	0.16±0.29 a	0.08±0.1 a	0.05±0.05 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	4.47E-15	42	100
159	Actinobacteria	0.07	0.0±0.0 a	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	0.16±0.2 a	0.39±0.55 a	0.0±0.0 a	0.0±0.0 a	CCB72805.1|Thermostable beta-glucosidase B [Streptomyces cattleya NRRL 8057 = DSM 46488]	1.13E-11	52.1	57.8
160	Actinobacteria	0.08	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.13±0.26 b	0.02±0.05 b	0.02±0.01 b	0.16±0.07 ab	0.33±0.21 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	1.74E-14	48.2	69.1
163	Betaproteobacteria	0.16	0.0±0.0 a	0.01±0.02 a	0.2±0.3 a	0.85±1.76 a	0.01±0.03 a	0.0±0.0 a	0.11±0.08 a	0.08±0.06 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	1.41E-32	82.3	98.4
167	Alphaproteobacteria	0.05	0.36±0.79 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	CCD02238.1|Beta-glucosidase A [Azospirillum brasilense Sp245]	2.65E-36	78.1	100
170	Deltaproteobacteria	0.11	0.01±0.02 a	0.0±0.0 a	0.39±0.43 a	0.31±0.69 a	0.04±0.03 a	0.05±0.03 a	0.08±0.07 a	0.05±0.03 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	4.23E-26	59.7	100
171	Actinobacteria	0.08	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	0.43±0.6 a	0.21±0.48 a	0.0±0.0 a	0.02±0.04 a	AGI88351.1|Beta-glucosidase [Streptomyces albus J1074]	4.49E-15	42	100
172	Actinobacteria	0.13	0.39±0.78 a	0.45±0.97 a	0.0±0.0 a	0.02±0.04 a	0.03±0.03 a	0.17±0.29 a	0.01±0.01 a	0.0±0.0 a	CAJ89567.1|putative beta-glucosidase [Streptomyces ambofaciens ATCC 23877]	3.68E-15	39.5	100
178	Actinobacteria	0.07	0.02±0.02 b	0.02±0.04 b	0.0±0.0 b	0.0±0.0 b	0.08±0.08 b	0.41±0.43 a	0.0±0.0 b	0.0±0.0 b	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	2.84E-13	44.6	69.1
179	Actinobacteria	0.09	0.47±0.92 a	0.0±0.0 a	0.01±0.02 a	0.01±0.02 a	0.17±0.14 a	0.04±0.04 a	0.01±0.03 a	0.01±0.01 a	AIR98549.1|Bifunctional beta-D-glucosidase/beta-D-fucosidase [Streptomyces glaucescens]	1.31E-15	62.9	46.1
181	Actinobacteria	0.04	0.33±0.75 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AGT83082.1|beta-glucosidase [Amycolatopsis mediterranei RB]	7.05E-16	44	100
182	Thermotogae	0.18	0.28±0.5 a	0.70±1.3 a	0.14±0.31 a	0.04±0.08 a	0.08±0.05 a	0.05±0.04 a	0.08±0.07 a	0.05±0.04 a	ABR31482.1|Beta-glucosidase [Thermosipho melanesiensis BI429]	2.18E-05	55.6	26.5
184	Actinobacteria	0.04	0.32±0.72 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCH29902.1|Thermostable beta-glucosidase B [Saccharothrix espanaensis DSM 44229]	6.06E-15	65.8	50
189	Betaproteobacteria	0.07	0.01±0.01 a	0.0±0.0 a	0.16±0.37 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.06±0.11 a	0.32±0.71 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	1.11E-33	84.1	100
197	Alphaproteobacteria	0.08	0.21±0.48 a	0.40±0.87 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	CCD00112.1|beta-glucosidase A [Azospirillum brasilense Sp245]	1.60E-22	53.8	100
198	Actinobacteria	0.10	0.0±0.0 a	0.06±0.14 a	0.03±0.07 a	0.32±0.47 a	0.03±0.04 a	0.02±0.01 a	0.14±0.05 a	0.19±0.11 a	AJE44123.1|beta-glucosidase [Streptomyces nodosus]	2.48E-12	45.8	59.3
201	Alphaproteobacteria	0.10	0.1±0.14 a	0.36±0.56 a	0.0±0.0 a	0.0±0.0 a	0.16±0.1 a	0.17±0.11 a	0.0±0.0 a	0.00±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	1.24E-18	52.3	100
207	Actinobacteria	0.22	0.10±0.22 a	0.43±0.96 a	0.13±0.2 a	0.85±1.29 a	0.01±0.02 a	0.0±0.0 a	0.11±0.11 a	0.11±0.13 a	AJE44123.1|beta-glucosidase [Streptomyces nodosus]	2.20E-12	45.8	59.3
220	Gammaproteobacteria	0.09	0.08±0.09 a	0.01±0.02 a	0.46±1.02 a	0.05±0.11 a	0.02±0.02 a	0.07±0.08 a	0.03±0.02 a	0.02±0.03 a	AFC17959.1|beta-glucosidase, partial [Pseudomonas sp. RBE6]	2.54E-11	36.9	100
222	Firmicutes	0.14	0.0±0.0 a	0.0±0.0 a	1.13±2.52 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	AEV69759.1|beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase [[Clostridium] clariflavum D	3.56E-13	39.2	81
230	Alphaproteobacteria	0.08	0.64±1.44 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	BAM89882.1|putative beta-glucosidase [Bradyrhizobium oligotrophicum S58]	5.82E-25	56.1	100
231	Alphaproteobacteria	0.13	0.02±0.04 a	0.0±0.0 a	1.0±2.25 a	0.01±0.02 a	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	3.78E-24	60	100
232	Gammaproteobacteria	0.13	0.0±0.0 a	0.0±0.0 a	0.26±0.41 a	0.73±1.42 a	0.0±0.0 a	0.0±0.0 a	0.02±0.02 a	0.03±0.02 a	CAA56282.1|beta-glucosidase [Pantoea agglomerans]	3.23E-18	55.4	100
238	Gammaproteobacteria	0.08	0.0±0.0 a	0.0±0.0 a	0.04±0.08 a	0.52±0.78 a	0.0±0.0 a	0.0±0.0 a	0.02±0.02 a	0.09±0.14 a	CAA56282.1|beta-glucosidase [Pantoea agglomerans]	1.24E-18	56.9	100
239	Gammaproteobacteria	0.08	0.0±0.0 a	0.0±0.0 a	0.02±0.45 a	0.52±0.72 a	0.0±0.0 a	0.0±0.0 a	0.03±0.02 a	0.07±0.11 a	CAA56282.1|beta-glucosidase [Pantoea agglomerans]	3.23E-18	53.1	98.5
243	Gammaproteobacteria	0.11	0.0±0.0 a	0.018±0.04 a	0.19±0.29 a	0.60±1.14 a	0.0±0.0 a	0.0±0.0 a	0.05±0.02 a	0.04±0.02 a	CAA56282.1|beta-glucosidase [Pantoea agglomerans]	4.43E-18	55.4	100
246	Actinobacteria	0.09	0.16±0.33 a	0.4±0.7 a	0.01±0.02 a	0.02±0.02 a	0.07±0.06 a	0.078±0.09 a	0.0±0.0 a	0.0±0.0 a	ACZ90607.1|Beta-glucosidase [Streptosporangium roseum DSM 43021]	1.64E-25	62.1	100
247	Alphaproteobacteria	0.10	0.0±0.0 a	0.0±0.0 a	0.43±0.97 a	0.0±0.0 a	0.03±0.03 a	0.02±0.03 a	0.1±0.1 a	0.18±0.37 a	BAI76508.1|beta-glucosidase [Azospirillum sp. B510]	2.95E-24	56.9	100
249	Actinobacteria	0.11	0.0±0.0 a	0.0±0.0 a	0.9±2.01 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AJC58446.1|Beta-glucosidase [Streptomyces sp. 769]	0.007943	33.3	73.7
254	Thermotogae	0.18	0.0±0.0 a	0.0±0.0 a	1.12±2.13 a	0.31±0.51 a	0.01±0.03 a	0.01±0.01 a	0.03±0.03 a	0.01±0.01 a	AFK06481.1|beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase [Mesotoga prima MesG1.Ag	1.61E-05	42.9	42.4
259	Actinobacteria	0.13	0.0±0.0 a	0.07±0.16 a	0.31±0.69 a	0.41±0.63 a	0.03±0.02 a	0.01±0.01 a	0.11±0.05 a	0.15±0.11 a	AGF66957.1|beta-glucosidase [Streptomyces hygroscopicus subsp. jinggangensis TL01]	1.14E-13	46.4	69.1
267	Actinobacteria	0.17	0.66±1.48 a	0.0±0.0 a	0.0±0.0 a	0.55±1.21 a	0.04±0.03 a	0.05±0.05 a	0.02±0.02 a	0.03±0.02 a	AIS02061.1|Bifunctional beta-D-glucosidase/beta-D-fucosidase [Streptomyces glaucescens]	7.98E-12	47.6	53.8
268	Alphaproteobacteria	0.12	0.0±0.0 a	0.05±0.1 a	0.07±0.13 a	0.81±1.32 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFL53886.1|beta-glucosidase Abg [Sinorhizobium fredii USDA 257]	0.005962	48	43.1
274	Deltaproteobacteria	0.12	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.7±1.32 a	0.12±0.09 a	0.04±0.04 a	0.04±0.05 a	0.02±0.02 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	5.56E-32	71.6	100
275	Actinobacteria	0.07	0.47±1.04 a	0.03±0.05 a	0.0±0.0 a	0.0±0.0 a	0.02±0.01 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	AIR98549.1|Bifunctional beta-D-glucosidase/beta-D-fucosidase [Streptomyces glaucescens]	6.87E-14	65.7	45.5
277	Deltaproteobacteria	0.06	0.45±0.71 a	0.03±0.05 a	0.0±0.0 a	0.0±0.0 a	0.02±0.01 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	ADO74619.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	4.93E-09	54.3	50.7
287	Alphaproteobacteria	0.10	0.0±0.0 a	0.0±0.0 a	0.57±1.28 a	0.0±0.0 a	0.02±0.04 a	0.02±0.03 a	0.09±0.09 a	0.12±0.24 a	BAI76508.1|beta-glucosidase [Azospirillum sp. B510]	2.54E-23	58.5	100
293	Bacteroidetes	0.08	0.04±0.08 a	0.33±0.69 a	0.0±0.0 a	0.0±0.0 a	0.03±0.04 a	0.06±0.09 a	0.04±0.03 a	0.13±0.11 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	1.28E-15	68.6	53.8
294	Unknown	0.05	0.35±0.76 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.04±0.04 a	0.05±0.05 a	0.0±0.0 a	0.0±0.0 a	AHI85742.1|beta-glucosidase [uncultured bacterium]	4.43E-26	63.1	100
297	Alphaproteobacteria	0.15	0.06±0.13 a	1.06±2.19 a	0.06±0.14 a	0.05±0.11 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	1.08E-23	60	100
301	Gammaproteobacteria	0.06	0.0±0.0 a	0.0±0.0 a	0.02±0.03 a	0.41±0.64 a	0.0±0.0 a	0.0±0.0 a	0.04±0.03 a	0.04±0.04 a	CAA56282.1|beta-glucosidase [Pantoea agglomerans]	4.43E-18	52.3	100
303	Betaproteobacteria	0.05	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.34±0.73 a	0.0±0.0 a	0.0±0.0 a	0.03±0.03 a	0.04±0.03 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	4.28E-34	84.1	100
306	Actinobacteria	0.06	0.46±0.9 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ACZ90607.1|Beta-glucosidase [Streptosporangium roseum DSM 43021]	5.82E-25	60.6	100
309	Alphaproteobacteria	0.12	0.2±0.43 a	0.19±0.32 a	0.0±0.0 a	0.43±0.71 a	0.07±0.04 a	0.09±0.08 a	0.0±0.0 a	0.0±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	2.17E-21	53.8	100
319	Verrucomicrobia	0.05	0.37±0.82 a	0.03±0.06 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.01±0.02 a	0.02±0.03 a	0.0±0.0 a	AHF92127.1|beta-glucosidase [Opitutaceae bacterium TAV5]	1.41E-20	62.5	100
326	Alphaproteobacteria	0.14	0.58±1.3 a	0.5±1.05 a	0.03±0.07 a	0.02±0.05 a	0.0±0.0 a	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	4.43E-22	57.8	98.5
330	Alphaproteobacteria	0.17	0.0±0.0 a	0.13±0.28 a	0.31±0.55 a	0.90±1.62 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ADO42989.1|Beta-glucosidase [Ketogulonicigenium vulgare Y25]	1.18E-06	59.3	33.3
332	Betaproteobacteria	0.07	0.0±0.0 a	0.02±0.04 a	0.31±0.53 a	0.15±0.16 a	0.0±0.0 a	0.0±0.0 a	0.05±0.02 a	0.0±0.0 a	ABE33903.1|Beta-glucosidase [Burkholderia xenovorans LB400]	1.65E-34	85.7	100
339	Bacteroidetes	0.46	3.71±8.27 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	7.73E-25	68.8	98.5
343	Firmicutes	0.07	0.0±0.0 a	0.0±0.0 a	0.6±1.32 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFH62665.1|beta-glucosidase [Paenibacillus mucilaginosus K02]	1.34E-06	55.6	36.5
344	Alphaproteobacteria	0.05	0.08±0.17 a	0.0±0.0 a	0.35±0.79 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ADO42989.1|Beta-glucosidase [Ketogulonicigenium vulgare Y25]	9.57E-06	59.3	33.3
353	Deltaproteobacteria	0.06	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.45±1.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEI64893.1|beta-glucosidase A [Myxococcus fulvus HW-1]	0.007939	57.1	16.5
377	Bacteroidetes	0.39	3.12±6.97 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	7.73E-25	67.7	100
379	Alphaproteobacteria	0.10	0.26±0.59 a	0.14±0.24 a	0.0±0.0 a	0.36±0.72 a	0.04±0.03 a	0.04±0.04 a	0.01±0.01 a	0.0±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	4.09E-21	56.9	100
380	Alphaproteobacteria	0.08	0.0±0.0 a	0.0±0.0 a	0.47±1.05 a	0.0±0.0 a	0.02±0.01 a	0.02±0.03 a	0.06±0.05 a	0.04±0.08 a	BAI76508.1|beta-glucosidase [Azospirillum sp. B510]	9.97E-24	58.5	100
398	Actinobacteria	0.04	0.0±0.0 a	0.0±0.0 a	0.34±0.75 a	0.01±0.03 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AIR98549.1|Bifunctional beta-D-glucosidase/beta-D-fucosidase [Streptomyces glaucescens]	2.40E-10	54.3	45.5
399	Deltaproteobacteria	0.12	0.88±1.97 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.04±0.09 a	0.01±0.01 a	0.01±0.01 a	0.0±0.0 a	CDK31020.1|Beta-glucosidase family enzyme [Candidatus Babela massiliensis]	6.09E-10	46	76.9
418	Alphaproteobacteria	0.06	0.0±0.0 a	0.0±0.0 a	0.46±1.02 a	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	3.49E-23	60	100
421	Alphaproteobacteria	0.09	0.36±0.8 a	0.35±0.7 a	0.0±0.0 a	0.02±0.05 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CBS91290.1|Beta-glucosidase A [Azospirillum lipoferum 4B]	2.35E-22	60	100
433	Gammaproteobacteria	0.06	0.35±0.77 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.06±0.09 a	0.06±0.08 a	0.0±0.0 a	0.0±0.0 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	1.08E-30	68.7	100
432	Deltaproteobacteria	0.10	0.76±1.69 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFC17959.1|beta-glucosidase, partial [Pseudomonas sp. RBE6]	9.81E-12	38.5	100
439	Deltaproteobacteria	0.04	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.32±0.71 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	8.62E-31	71.6	100
440	Deltaproteobacteria	0.04	0.0±0.0 a	0.0±0.0 a	0.32±0.71 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	8.31E-32	73.1	100
443	Firmicutes	0.05	0.0±0.0 a	0.0±0.0 a	0.43±0.95 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEV69759.1|beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase [[Clostridium] clariflavum D	1.68E-12	39.2	81
449	Gammaproteobacteria	0.07	0.0±0.0 a	0.02±0.04 a	0.33±0.59 a	0.22±0.44 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AAZ50390.1|beta-glucosidase, partial [Shewanella sp. G5]	4.53E-14	51.2	70.7
451	Deltaproteobacteria	0.05	0.0±0.0 a	0.0±0.0 a	0.32±0.71 a	0.01±0.03 a	0.0±0.0 a	0.0±0.0 a	0.02±0.02 a	0.01±0.01 a	ADO74619.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	8.37E-10	61.3	47.7
529	Gammaproteobacteria	0.13	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	1.0±2.21 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ABD82858.1|putative retaining b-glycosidase [Saccharophagus degradans 2-40]	1.63E-07	55.6	37
537	Alphaproteobacteria	0.11	0.0±0.0 a	0.1±0.22 a	0.12±0.2 a	0.64±1.02 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ADO42989.1|Beta-glucosidase [Ketogulonicigenium vulgare Y25]	1.56E-06	59.3	33.3
540	Firmicutes	0.04	0.0±0.0 a	0.0±0.0 a	0.31±0.70 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEV69759.1|beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase [[Clostridium] clariflavum D	1.46E-11	39.2	81
616	Bacteroidetes	0.18	1.45±3.25 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	4.09E-25	66.2	100
627	Gammaproteobacteria	0.05	0.43±0.97 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFC17959.1|beta-glucosidase, partial [Pseudomonas sp. RBE6]	7.14E-12	40	100
681	Actinobacteria	0.05	0.41±0.91 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.01±0.03 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	AGS72850.1|beta-glucosidase [Streptomyces collinus Tu 365]	3.23E-14	48.2	69.1
759	Deltaproteobacteria	0.05	0.42±0.92 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	ADO73143.1|Beta-glucosidase A [Stigmatella aurantiaca DW4/3-1]	7.22E-27	64.2	100
833	Deltaproteobacteria	0.04	0.34±0.73 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDK31020.1|Beta-glucosidase family enzyme [Candidatus Babela massiliensis]	4.44E-10	46	76.9
1391	Bacteroidetes	0.05	0.44±0.97 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	4.09E-25	66.2	100
1605	Bacteroidetes	0.04	0.36±0.8 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	3.07E-25	67.7	100
1804	Bacteroidetes	0.04	0.32±0.72 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFC17957.1|beta-glucosidase, partial [Flavobacterium sp. RBE3]	7.14E-24	66.2	100

Supplementary Table 4: Overview of taxonomical assignment of most abundant OTUs of fungal β-glucosidase genes from GH1 family, their abundance and districution among L and H horizons and among DNA and RNA in summer and winter. Abundance data are expressed as means (%) and standard deviations from six different sites. Statastically significant differences in relative abundance among HLR, HZR, LLR, LZR, HLD, HZD, LLD and LZD (ANOVA followed by TuKey Post-hoc) are indicated by different letters.

OTUs	Assignment	Mean Abundance (%)	HLR (%)	HZR (%)	LLR (%)	LZR (%)	HLD (%)	HZD (%)	LLD (%)	LZD (%)	Best Hit	E value	Similarity (%)	Coverage (%)
CL0	Ascomycota	33.48	66.16±27.50 ab	89.26±5.93 a	54.11±21.75 bc	24.04±20.18 cd	18.54±18.69 d	9.97±7.61 d	1.91±3.19 d	3.85±5.3 d	XP_365969.1|hypothetical protein MGG_10189 [Magnaporthe oryzae 70-15]	1.99E-87	82.4	100
CL1	Ascomycota	7.18	2.51±3.13 cde	0.76±0.90 e	1.47±2.19 de	0.38±0.74 e	9.74±5.79 abc	8.92±2.16 bcd	16.11±5.46 ab	17.55±8.19 a	EAA65642.1|hypothetical protein AN0812.2 [Aspergillus nidulans FGSC A4]	3.15E-71	71.1	45.8
CL2	Ascomycota	5.43	2.28±4.13 c	0.36±0.89 c	2.37±5.79 c	0.69±1.69 c	6.15±3.87 bc	6.51±3.36 bc	9.58±3.22 ab	15.50±5.04 a	CBX97760.1|hypothetical protein LEMA_P091690.1 [Leptosphaeria maculans JN3]	2.55E-67	75.3	44.8
CL3	Ascomycota	4.44	3.91±6.06 a	0.36±0.89 a	3.87±9.48 a	0.60±1.29 a	6.77±3.72 a	4.10±1.15 a	8.27±2.74 a	7.65±4.65 a	BAE63197.1|unnamed protein product [Aspergillus oryzae RIB40]	2.01E-63	62.2	100
CL5	Ascomycota	1.40	0.13±0.31 a	0.51±0.65 a	0.0±0.0 a	10.58±22.19 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	5.63E-88	80.4	100
CL6	Ascomycota	3.72	0.06±0.16 b	0.36±0.89 b	0.0±0.0 b	0.06±0.15 b	7.34±7.92 b	16.78±7.35 a	2.84±1.45 b	2.28±2.37 b	CAK47813.1|unnamed protein product [Aspergillus niger]	3.11E-95	91.8	42.7
CL7	Ascomycota	2.15	0.06±0.16 b	1.22±1.23 b	0.32±0.54 b	9.10±5.96 a	0.0±0.0 b	0.24±0.38 b	3.78±3.25 b	2.47±2.76 b	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	4.63E-90	85.8	100
CL8	Ascomycota	2.28	0.0±0.0 a	0.18±0.44 a	4.17±10.21 a	12.98±19.07 a	0.0±0.0 a	0.0±0.0 a	0.40±0.49 a	0.47±1.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	1.17E-90	87.2	100
CL9	Ascomycota	2.80	0.58±0.70 bc	0.36±0.89 c	0.26±0.42 c	0.13±0.21 c	5.14±3.86 b	10.34±4.97 a	2.84±2.48 bc	2.75±1.96 bc	EAA63677.1|hypothetical protein AN3106.2 [Aspergillus nidulans FGSC A4]	1.91E-79	85.2	100
CL12	Ascomycota	2.43	2.15±2.19 a	0.83±2.02 a	0.64±0.84 a	0.0±0.0 a	5.05±4.71 a	3.36±2.39 a	2.71±1.95 a	4.67±4.49 a	EAA63677.1|hypothetical protein AN3106.2 [Aspergillus nidulans FGSC A4]	1.80E-81	80.5	100
CL14	Ascomycota	1.52	0.0±0.0 a	0.0±0.0 a	0.11±0.28 a	12.06±26.78 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	2.13E-91	82.4	100
CL15	Ascomycota	2.55	0.38±0.53 b	0.54±0.87 b	0.27±0.43 b	0.0±0.0 b	11.33±9.32 a	4.97±3.64 ab	1.13±1.55 b	1.83±2.42 b	CEF83492.1|unnamed protein product [Fusarium graminearum]	1.39E-72	83.2	52.8
CL16	Ascomycota	1.67	0.84±2.05 bc	0.99±1.49 bc	0.32±0.79 bc	0.15±0.38 c	1.38±1.95 bc	1.54±1.19 bc	3.32±1.53 ab	4.79±3.30 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	2.78E-68	74.7	46.9
CL17	Ascomycota	1.45	0.13±0.32 c	0.0±0.0 c	0.0±0.0 c	0.08±0.19 c	1.37±1.13 bc	3.20±0.89 ab	4.14±2.75 a	2.71±1.35 ab	CCD55258.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	2.57E-78	84.6	100
CL18	Ascomycota	1.27	0.76±1.37 bc	0.0±0.0 c	0.22±0.53 c	0.19±0.47 c	1.20±1.47 bc	1.07±1.24 bc	2.79±2.15 ab	3.92±1.24 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	2.99E-68	71.6	100
CL19	Ascomycota	1.29	2.15±2.72 a	0.45±0.87 a	0.65±1.58 a	0.15±0.38 a	2.01±3.32 a	1.19±1.39 a	1.81±1.57 a	1.93±2.07 a	ADM15720.1|beta-glucosidase [Paecilomyces sp. J18]	4.16E-74	76	45.2
CL20	Ascomycota	0.89	0.50±0.82 a	0.0±0.0 a	0.43±1.05 a	0.15±0.38 a	0.29±0.48 a	0.39±0.60 a	2.43±2.66 a	2.91±5.26 a	AAL34084.2|beta-glucosidase 1 [Rasamsonia emersonii]	1.30E-74	72.6	40.6
CL21	Ascomycota	1.26	0.66±0.91 a	0.0±0.0 a	0.0±0.0 a	0.11±0.26 a	2.37±2.23 a	3.02±3.42 a	1.05±0.49 a	2.84±2.19 a	AAQ21384.1|beta-glucosidase 2 [Trichoderma viride]	4.72E-06	71.4	36.8
CL22	Ascomycota	0.72	0.0±0.0 b	0.17±0.40 ab	0.34±0.84 ab	0.0±0.0 b	0.54±0.64 ab	0.21±0.50 ab	2.27±1.58 a	2.26±2.72 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	2.33E-77	73.6	100
CL25	Ascomycota	1.82	0.57±1.07 a	1.99±2.66 a	1.49±1.78 a	10.51±23.36 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	2.12E-88	86.7	55.7
CL27	Ascomycota	0.65	0.26±0.63 b	0.0±0.0 b	0.22±0.53 b	0.0±0.0 ab	0.67±1.11 ab	0.18±0.44 b	2.57±3.38 a	1.30±1.19 ab	BAE63197.1|unnamed protein product [Aspergillus oryzae RIB40]	3.50E-62	60.8	100
CL29	Ascomycota	0.71	0.13±0.32 a	0.0±0.0 a	0.32±0.79 a	0.0±0.0 a	1.99±2.40 a	0.98±0.59 a	1.04±0.91 a	1.20±1.46 a	CCD55258.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	2.66E-32	91.1	94.9
CL30	Ascomycota	1.21	0.76±1.86 a	0.0±0.0 a	8.95±15.61 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	XP_322216.1|hypothetical protein ((AB003109) beta-glucosidase [Humicola grisea var. thermoidea]	2.99E-87	79.1	100
CL31	Ascomycota	0.70	0.26±0.63 a	0.0±0.0 a	0.32±0.79 a	0.14±0.24 a	1.30±0.91 a	1.31±1.99 a	1.64±1.10 a	0.60±0.99 a	BAE63197.1|unnamed protein product [Aspergillus oryzae RIB40]	2.17E-64	63.5	100
CL32	Ascomycota	1.02	0.0±0.0 a	0.0±0.0 a	5.09±9.82 a	3.04±2.58 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	8.29E-93	84.5	100
CL36	Ascomycota	0.42	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.30±0.33 b	0.0±0.0 b	1.83±1.18 a	1.21±1.23 ab	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	2.55E-78	76.4	100
CL38	Ascomycota	0.32	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.10±0.24 b	0.0±0.0 b	1.46±1.04 a	1.03±0.65 b	AII80277.1|beta-glucosidase [Humicola insolens]	3.96E-77	80.3	46.1
CL39	Ascomycota	0.34	0.0±0.0 a	0.0±0.0 a	0.11±0.26 a	0.08±0.19 a	0.29±0.71 a	0.40±0.47 a	0.96±1.0 a	0.85±1.56 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	6.17E-82	75.7	100
CL40	Ascomycota	0.62	4.92±12.06 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFO54488.1|beta-glucosidase, partial [Stachybotrys microspora]	2.35E-43	85.3	73.5
CL41	Ascomycota	0.50	0.0±0.0 a	0.0±0.0 a	3.20±7.82 a	0.76±1.87 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.83E-94	86.5	100
CL42	Ascomycota	0.37	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.23±0.57 b	0.0±0.0 b	2.4±2.13 a	0.31±0.33 b	CCD55258.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	2.56E-88	85.9	100
CL44	Ascomycota	0.65	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	2.65±5.38 a	2.26±3.08 a	0.24±0.59 a	0.04±0.10 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	4.34E-85	84.4	46.7
CL47	Ascomycota	0.38	0.11±0.28 ab	0.0±0.0 b	0.11±0.26 ab	0.0±0.0 b	0.87±0.68 ab	0.0±0.0 b	0.77±0.40 ab	1.17±1.62 a	CBX97760.1|hypothetical protein LEMA_P091690.1 [Leptosphaeria maculans JN3]	9.07E-78	70.9	100
CL48	Ascomycota	0.36	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.13±0.21 b	0.24±0.58 ab	0.47±0.54 ab	1.50±1.06 a	0.58±1.02 ab	CCD55258.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	2.83E-79	85.2	100
CL49	Ascomycota	0.26	0.0±0.0 a	0.18±0.44 a	0.0±0.0 a	1.52±2.94 a	0.0±0.0 a	0.0±0.0 a	0.13±0.21 a	0.20±0.32 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	1.17E-90	87.2	100
CL50	Ascomycota	0.43	3.42±7.99 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.23E-89	82.4	100
CL51	Ascomycota	0.36	0.0±0.0 a	0.0±0.0 a	2.76±4.99 a	0.15±0.26 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	6.84E-94	85.8	100
CL52	Ascomycota	0.45	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.48±1.17 a	3.16±7.44 a	0.0±0.0 a	0.0±0.0 a	AII80277.1|beta-glucosidase [Humicola insolens]	6.41E-86	90.4	44.8
CL54	Ascomycota	0.21	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.19±0.47 a	0.59±1.02 a	0.27±0.27 a	0.66±1.038 a	ABN50090.1|beta-1,4-glucosidase [Trichoderma harzianum]	1.04E-88	89.1	100
CL58	Ascomycota	0.21	0.06±0.16 b	0.0±0.0 b	0.0±0.0 b	0.04±0.09 b	0.19±0.47 ab	0.12±0.29 ab	0.90±0.57 a	0.37±0.51 ab	BAE63197.1|unnamed protein product [Aspergillus oryzae RIB40]	1.99E-71	68.9	100
CL59	Ascomycota	0.26	0.0±0.0 a	0.0±0.0 a	0.34±0.84 a	0.0±0.0 a	0.33±0.58 a	0.71±0.98 a	0.39±0.34 a	0.33±0.32 a	ABN50090.1|beta-1,4-glucosidase [Trichoderma harzianum]	2.61E-75	88.9	52.2
CL60	Ascomycota	0.47	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	1.45±2.16 a	2.25±3.25 a	0.0±0.0 a	0.05±0.13 a	CAK47813.1|unnamed protein product [Aspergillus niger]	3.25E-89	84.9	44.8
CL61	Ascomycota	0.20	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.10±0.24 a	0.26±0.40 a	0.92±1.12 a	0.30±0.49 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	8.30E-77	74.3	100
CL63	Ascomycota	0.27	1.31±2.73 a	0.26±0.64 a	0.46±0.57 a	0.15±0.26 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.45E-91	83.1	100
CL65	Ascomycota	0.22	0.06±0.16 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.63±0.69 a	0.12±0.29 a	0.29±0.24 a	0.65±0.76 a	EAA63677.1|hypothetical protein AN3106.2 [Aspergillus nidulans FGSC A4]	4.91E-80	85.9	100
CL67	Ascomycota	0.25	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.04±0.09 a	0.52±0.81 a	0.61±0.75 a	0.39±0.23 a	0.41±0.64 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	2.25E-66	72.6	45.6
CL68	Ascomycota	0.29	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.63±0.59 a	0.85±1.28 a	0.24±0.40 a	0.60±0.74 a	AAQ21384.1|beta-glucosidase 2 [Trichoderma viride]	4.72E-06	71.4	36.8
CL70	Ascomycota	0.22	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	1.52±3.71 a	0.0±0.0 a	0.0±0.0 a	0.11±0.26 a	0.11±0.26 a	AEO57459.1|glycoside hydrolase family 1 protein [Myceliophthora thermophila ATCC 42464]	3.15E-88	82.4	100
CL74	Ascomycota	0.24	0.0±0.0 a	0.0±0.0 a	0.80±1.96 a	1.14±1.34 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	4.06E-88	81.1	100
CL76	Ascomycota	0.11	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.21±0.50 a	0.10±0.25 a	0.53±1.30 a	0.04±0.11 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	9.55E-87	85	49.4
CL78	Ascomycota	0.14	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.10±0.24 a	0.0±0.0 a	0.61±1.13 a	0.38±0.45 a	CBX97760.1|hypothetical protein LEMA_P091690.1 [Leptosphaeria maculans JN3]	1.60E-74	78.1	44.2
CL79	Ascomycota	0.14	0.0±0.0 a	0.0±0.0 a	0.11±0.27 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.79±0.98 a	0.23±0.37 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	1.45E-75	73	100
CL80	Ascomycota	0.08	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.45±1.10 a	0.17±0.28 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.24E-85	84.4	45.8
CL81	Ascomycota	0.09	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.67±1.43 a	0.0±0.0 a	0.0±0.0 a	0.05±0.13 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	1.25E-89	85.8	100
CL82	Ascomycota	0.16	0.0±0.0 b	0.0±0.0 b	0.11±0.28 ab	1.13±1.4 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	1.46E-72	72.7	78.6
CL83	Ascomycota	0.14	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.76±1.29 a	0.333±0.66 a	AII80277.1|beta-glucosidase [Humicola insolens]	3.75E-95	86.5	100
CL85	Ascomycota	0.13	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.78±0.96 a	0.0±0.0 b	0.0±0.0 b	0.15±0.36 ab	0.14±0.34 ab	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	3.03E-90	86.5	100
CL87	Ascomycota	0.27	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.57±1.14 a	1.60±3.02 a	0.0±0.0 a	0.0±0.0 a	ADM15720.1|beta-glucosidase [Paecilomyces sp. J18]	3.94E-07	53.8	20.2
CL88	Ascomycota	0.14	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.08±0.18 a	0.48±1.19 a	0.12±0.29 a	0.19±0.36 a	0.23±0.32 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	3.89E-91	82.5	49.1
CL92	Ascomycota	0.18	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.11±0.267 ab	1.31±1.91 a	0.04±0.10 a	0.0±0.0 a	BAE63197.1|unnamed protein product [Aspergillus oryzae RIB40]	1.24E-73	70.3	100
CL93	Ascomycota	0.12	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.21±0.5 a	0.0±0.0 a	0.49±0.88 a	0.25±0.38 a	CCD55258.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	1.93E-67	82.2	60.4
CL94	Ascomycota	0.12	0.0±0.0 a	0.11±0.26 a	0.11±0.26 a	0.04±0.09 a	0.10±0.24 a	0.0±0.0 a	0.26±0.54 a	0.35±0.45 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.89E-90	81.3	49.1
CL103	Ascomycota	0.07	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.38±0.92 a	0.0±0.0 a	0.0±0.0 a	0.05±0.13 a	0.16±0.40 a	XP_322216.1|hypothetical protein ((AB003109) beta-glucosidase [Humicola grisea var. thermoidea]	6.09E-97	89.9	100
CL104	Ascomycota	0.11	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.11±0.27 a	0.0±0.0 a	0.61±0.88 a	0.15±0.25 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.73E-89	89.3	46
CL106	Ascomycota	0.17	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.67±1.08 a	0.46±0.86 a	0.24±0.41 a	0.0±0.0 a	XP_001263204.1|beta-glucosidase [Neosartorya fischeri NRRL 181]	1.41E-29	85.2	91.5
CL108	Ascomycota	0.11	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.10±0.24 a	0.10±0.25 a	0.56±0.72 a	0.13±0.34 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	1.34E-78	75.7	100
CL112	Ascomycota	0.05	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.37±0.9 a	0.04±0.10 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	3.52E-86	85.7	45.8
CL114	Ascomycota	0.13	0.0±0.0 a	0.0±0.0 a	0.43±1.06 a	0.0±0.0 a	0.0±0.0 a	0.14±0.34 a	0.24±0.41 a	0.21±0.32 a	AGC30579.1|glycoside hydrolase family 1 beta-glucosidase [Phialophora sp. G5]	9.25E-96	87.8	100
CL116	Ascomycota	0.08	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.64±1.09 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	3.69E-90	85.1	100
CL120	Ascomycota	0.21	0.51±1.23 a	0.16±0.26 a	0.23±0.57 a	0.85±1.87 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.05E-91	83.8	100
CL126	Ascomycota	0.10	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.30±0.51 a	0.0±0.0 a	0.12±0.20 a	0.37±0.51 a	CCD55258.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	5.04E-89	87.2	100
CL127	Ascomycota	0.12	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.91±1.54 a	0.0±0.0 a	0.06±0.13 a	BAE63197.1|unnamed protein product [Aspergillus oryzae RIB40]	1.84E-74	68.9	100
CL129	Ascomycota	0.10	0.51±1.24 a	0.0±0.0 a	0.0±0.0 a	0.26±0.36 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	1.51E-93	87.2	100
CL132	Ascomycota	0.06	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.47±0.67 a	0.0±0.0 a	XP_322216.1|hypothetical protein ((AB003109) beta-glucosidase [Humicola grisea var. thermoidea]	1.20E-66	64.9	48.1
CL133	Ascomycota	0.08	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.61±1.5 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	3.23E-91	87.2	100
CL134	Ascomycota	0.07	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.40±0.98 a	0.14±0.24 a	AII80277.1|beta-glucosidase [Humicola insolens]	3.77E-94	86.5	100
CL135	Ascomycota	0.15	0.0±0.0 a	0.0±0.0 a	0.94±2.30 a	0.28±0.49 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	6.73E-91	86.5	100
CL137	Ascomycota	0.07	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.53±0.92 a	0.0±0.0 a	XP_322216.1|hypothetical protein ((AB003109) beta-glucosidase [Humicola grisea var. thermoidea]	2.33E-89	81.8	100
CL138	Ascomycota	0.08	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.10±0.24 a	0.0±0.0 a	0.510±1.25 a	0.0±0.0 a	CCD55258.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	1.84E-77	85.2	100
CL139	Ascomycota	0.17	1.33±3.25 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFO54488.1|beta-glucosidase, partial [Stachybotrys microspora]	4.39E-93	87.2	100
CL142	Ascomycota	0.07	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.45±1.09 a	0.09±0.23 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	3.63E-84	79.1	100
CL144	Ascomycota	0.12	0.0±0.0 b	0.0±0.0 b	0.19±0.29 ab	0.04±0.09 b	0.0±0.0 b	0.65±0.76 a	0.0±0.0 b	0.11±0.26 ab	AGC30579.1|glycoside hydrolase family 1 beta-glucosidase [Phialophora sp. G5]	2.13E-34	79.4	92.6
CL145	Ascomycota	0.09	0.0±0.0 a	0.0±0.0 a	0.23±0.57 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.13±0.21 a	0.36±0.47 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.51E-91	83.8	49.1
CL147	Ascomycota	0.07	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.18±0.44 a	0.38±0.49 a	0.0±0.0 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	8.99E-78	75.7	100
CL149	Ascomycota	0.09	0.06±0.16 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.11±0.27 a	0.37±0.9 a	0.0±0.0 a	0.2±0.30 a	ACZ34300.1|beta-glucosidase II [Trichoderma longibrachiatum]	9.72E-06	43.9	60.3
CL150	Ascomycota	0.08	0.0±0.0 a	0.0±0.0 a	0.65±1.59 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CBX97760.1|hypothetical protein LEMA_P091690.1 [Leptosphaeria maculans JN3]	1.40E-83	82.2	44.2
CL152	Ascomycota	0.07	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.30±0.51 a	0.10±0.25 a	0.15±0.36 a	0.04±0.11 a	BAE63197.1|unnamed protein product [Aspergillus oryzae RIB40]	1.25E-81	81.1	100
CL154	Ascomycota	0.09	0.44±0.69 a	0.0±0.0 a	0.11±0.28 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.13±0.21 a	0.0±0.0 a	XP_322216.1|hypothetical protein ((AB003109) beta-glucosidase [Humicola grisea var. thermoidea]	2.04E-97	90.5	100
CL155	Ascomycota	0.09	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.30±0.51 a	0.31±0.76 a	0.08±0.20 a	0.0±0.0 a	CBX97760.1|hypothetical protein LEMA_P091690.1 [Leptosphaeria maculans JN3]	5.86E-88	84.4	47
CL160	Ascomycota	0.09	0.76±1.85 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	9.18E-98	91.9	100
CL200	Ascomycota	0.04	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.33±0.58 a	0.0±0.0 a	ABN50090.1|beta-1,4-glucosidase [Trichoderma harzianum]	1.36E-68	91.7	52.6
CL205	Ascomycota	0.06	0.0±0.0 a	0.0±0.0 a	0.34±0.84 a	0.1±0.25 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ADM15720.1|beta-glucosidase [Paecilomyces sp. J18]	1.84E-78	73	100
CL206	Ascomycota	0.11	0.0±0.0 a	0.26±0.63 a	0.0±0.0 a	0.59±0.98 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	6.60E-90	84.5	100
CL210	Ascomycota	0.16	0.0±0.0 a	0.0±0.0 a	0.11±0.28 a	1.16±2.85 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	2.98E-84	85.1	100
CL213	Ascomycota	0.06	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.49±0.8 a	0.0±0.0 a	0.0±0.0 a	AFO54488.1|beta-glucosidase, partial [Stachybotrys microspora]	4.76E-82	77.7	100
CL214	Ascomycota	0.09	0.0±0.0 a	0.0±0.0 a	0.70±1.17 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.83E-94	86.5	100
CL223	Ascomycota	0.08	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.50±0.79 a	0.0±0.0 a	0.11±0.26 a	BAE63197.1|unnamed protein product [Aspergillus oryzae RIB40]	2.76E-83	78.4	100
CL237	Ascomycota	0.04	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.30±0.74 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD54136.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	9.91E-93	87.8	100
CL241	Ascomycota	0.04	0.35±0.57 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	2.52E-94	85.1	100
CL268	Ascomycota	0.04	0.0±0.0 a	0.0±0.0 a	0.34±0.84 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	4.40E-89	82.4	100
CL295	Ascomycota	0.06	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.47±0.73 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCT76035.1|probable beta-glucosidase [Fusarium fujikuroi IMI 58289]	1.13E-09	54.3	68.6
CL340	Ascomycota	0.06	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.46±1.13 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEO69521.1|glycoside hydrolase family 1 protein [Thielavia terrestris NRRL 8126]	9.70E-80	77.2	48.2
CL343	Ascomycota	0.06	0.0±0.0 a	0.0±0.0 a	0.47±1.15	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CDP32570.1|Putative Glycoside Hydrolase Family 1 [Podospora anserina S mat+]	1.45E-91	83.1	100
CL345	Ascomycota	0.06	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.48±1.17 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AAQ21384.1|beta-glucosidase 2 [Trichoderma viride]	4.84E-08	85.7	36.8
CL435	Ascomycota	0.04	0.0±0.0 a	0.33±0.8 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD55258.1|glycoside hydrolase family 1 protein [Botrytis cinerea T4]	5.32E-84	81.9	100

Supplementary Table 5: Overview of taxonomical assignment of most abundant OTUs of bacterial β-glucosidase genes from GH3 family, their abundance and districution among L and H horizons and among DNA and RNA in summer and winter. Abundance data are expressed as means (%) and standard deviations from six different sites. Statastically significant differences in relative abundance among HLR, HZR, LLR, LZR, HLD, HZD, LLD and LZD (ANOVA followed by TuKey Post-hoc) are indicated by different letters.

OTUs	Assignment	Mean Abundance (%)	HLR (%)	HZR (%)	LLR (%)	LZR (%)	HLD (%)	HZD (%)	LLD (%)	LZD (%)	Best Hit	E value	Similarity (%)	Coverage (%)
CL1	Firmicutes	57.20	49.73±7.67 c	52.88±11.87 bc	60.38±6.11 abc	50.85±15.54 c	43.52±21.25 c	51.85±8.074 c	73.58±4.18 ab	74.77±8.9 a	AIQ12017.1|beta-glucosidase [Paenibacillus durus]	0.003328	41.5	64.1
CL5	Acidobacteria	10.25	18.67±11.95 ab	13.86±18.88 ab	1.82±0.94 b	1.22±1.0 b	24.08±19.44 a	17.53±8.91 ab	1.49±1.15 b	3.34±5.34 b	ACO33751.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	1.15E-55	80.4	100
CL27	Firmicutes	1.93	2.69±1.4 a	3.34±3.17 a	1.61±2.82 a	0.73±0.36 a	1.80±0.83 a	1.88±0.47 a	1.38±0.49 a	2.02±0.54 a	AIQ12017.1|beta-glucosidase [Paenibacillus durus]	0.003328	41.5	64.1
CL31	GammaProteoBacteria	2.66	2.02±2.5 a	1.78±3.24 a	3.30±2.28 a	7.23±11.45 a	2.15±2.08 a	4.27±2.92 a	0.09±0.09 a	0.43±0.72 a	CDF62508.1|putative beta-glucosidase [Xanthomonas fuscans subsp. fuscans]	0.000659	38.9	65.5
CL46	Firmicutes	1.09	1.48±0.34 a	1.25±0.31 ab	1.40±0.37 a	1.08±0.65 ab	0.71±0.43 b	0.94±0.20 ab	0.89±0.15 ab	0.98±0.16 ab	AIQ12017.1|beta-glucosidase [Paenibacillus durus]	0.003328	41.5	64.1
CL107	Acidobacteria	0.37	0.17±0.16 b	0.34±0.59 ab	0.1±0.15 b	0.0±0.0 b	1.33±1.33 a	0.63±0.94 ab	0.21±0.18 ab	0.18±0.13 ab	ACO33751.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	1.24E-56	80.4	100
CL113	Actinobacteria	0.33	0.51±1.16 a	1.87±4.09 a	0.09±0.15 a	0.01±0.03 a	0.0±0.0 a	0.0±0.0 a	0.10±0.11 a	0.05±0.05 a	CDR04858.1|Beta-glucosidase [Streptomyces iranensis]	0.005933	37.5	70.2
CL118	Fermicutes	0.45	0.14±0.19 b	0.13±0.16 b	0.30±0.45 b	0.35±0.46 b	1.42±1.15 a	0.98±0.57 ab	0.1±0.1 b	0.17±0.32 b	ADB09184.1|Putative Beta-glucosidase [Bifidobacterium dentium Bd1]	0.006723	52.2	62.2
CL120	Acidobacteria	0.25	0.15±0.16 bc	0.09±0.07 c	0.09±0.11 c	0.09±0.16 c	0.12±0.09 bc	0.22±0.16 bc	0.79±0.47 a	0.51±0.27 ab	ACO33751.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	2.06E-45	70.6	100
CL127	GammaProteoBacteria	0.56	1.95±3.81 a	0.47±0.78 a	0.39±0.85 a	1.53±3.56 a	0.0±0.0 a	0.0±0.0 a	0.10±0.10 a	0.03±0.02 a	AEL06905.1|glucan 1,4-beta-glucosidase [Xanthomonas campestris pv. raphani 756C]	0.009981	57.9	39.6
CL133	AlphaProteoBacteria	0.93	0.0±0.0 b	1.12±2.74 ab	4.87±4.86 a	1.42±1.54 ab	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	BAI98612.1|beta-glucosidase [Sphingobium japonicum UT26S]	0.009173	53.6	66.7
CL137	BetaProteoBacteria	0.34	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.99±0.63 ab	1.50±1.73 a	0.07±0.09 b	0.13±0.08 b	ACB64803.1|Beta-glucosidase [Burkholderia ambifaria MC40-6]	0.001361	41	84.8
CL151	GammaProteoBacteria	0.19	0.0±0.0 a	1.48±3.64 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AIR68391.1|beta-glucosidase [Cedecea neteri]	0.001367	58.3	60
CL176	Actinobacteria	0.30	0.22±0.51 a	0.13±0.19 a	0.0±0.0 a	0.0±0.0 a	1.30±2.39 a	0.67±1.30 a	0.03±0.03 a	0.03±0.04 a	AEU36797.1|Beta-glucosidase [Granulicella mallensis MP5ACTX8]	1.94E-14	65.1	97.7
CL177	GammaProteoBacteria	0.26	1.15±2.45 a	0.38±0.92 a	0.0±0.0 a	0.0±0.0 a	0.17±0.33 a	0.40±0.79 a	0.01±0.02 a	0.0±0.0 a	NP_639159.1|beta-glucosidase [Xanthomonas campestris pv. campestris str. ATCC 33913]	7.81E-05	48.5	78.6
CL184	Thermotogae	0.19	0.59±1.02 a	0.09±0.14 a	0.01±0.03 a	0.1±0.23 a	0.0±0.0 a	0.03±0.04 a	0.44±0.23 a	0.26±0.09 a	AEH51751.1|beta-glucosidase [Thermotoga thermarum DSM 5069]	0.003562	75	31.6
CL187	GammaProteoBacteria	0.30	0.02±0.036 a	0.025±0.06 a	0.47±0.89 a	1.4±2.6 a	0.04±0.04 a	0.09±0.05 a	0.22±0.19 a	0.13±0.09 a	CCH12773.1|Glucan 1,4-beta-glucosidase [Stenotrophomonas maltophilia D457]	0.003695	50	34.4
CL201	AlphaProteoBacteria	0.37	0.042±0.10 a	2.88±7.07 a	0.0±0.0 a	0.01±0.03 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	NP_420564.1|beta-D-glucosidase [Caulobacter crescentus CB15]	0.00535	34.4	94.1
CL206	GammaProteoBacteria	0.51	0.13±0.24 a	0.04±0.05 a	1.70±0.83 b	2.25±1.86 b	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ACD57382.1|periplasmic beta-glucosidase [Xanthomonas oryzae pv. oryzae PXO99A]	0.004151	40	44.1
CL210	BetaProteoBacteria	0.31	0.014±0.03 a	0.43±0.76 a	1.91±3.04 b	0.017±0.042 a	0.07±0.14 a	0.02±0.02 a	0.0±0.0 a	0.0±0.0 a	ACA92476.1|Beta-glucosidase [Burkholderia cenocepacia MC0-3]	0.00108	53.6	59.6
CL212	Acidobacteria	0.12	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.15±0.32 a	0.0±0.0 a	0.03±0.07 a	0.65±1.33 a	0.14±0.26 a	ACO33751.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	7.72E-55	77.5	100
CL216	Unknown	0.42	1.90±2.35 a	0.41±0.38 b	0.53±0.94 b	0.12±0.17 b	0.41±0.99b	0.0±0.0 b	0.01±0.02 b	0.0±0.0 b	ABB51613.1|beta-glucosidase [uncultured bacterium]	0.003556	39.5	97.4
CL221	Actinobacteria	0.12	0.0±0.0 a	0.69±1.68 a	0.0±0.0 a	0.02±0.04 a	0.0±0.0 a	0.0±0.0 a	0.05±0.08 a	0.18±0.36 a	AIT82164.1|beta-glucosidase [Novosphingobium pentaromativorans US6-1]	0.001503	54.2	40
CL220	AlphaProteoBacteria	0.38	0.0±0.0 b	0.22±0.55 b	1.12±1.81 a	1.44±3.52 a	0.0±0.0 b	0.0±0.0 b	0.21±0.16 b	0.08±0.07 b	ABL82765.1|Beta-glucosidase [Nocardioides sp. JS614]	0.002346	60	39.5
CL229	Actinobacteria	0.31	0.01±0.02 b	0.0±0.0 b	0.60±1.40 a	1.81±4.43 a	0.0±0.0 b	0.0±0.0 b	0.04±0.05 b	0.02±0.03 b	CCA55499.1|Beta-glucosidase [Streptomyces venezuelae ATCC 10712]	0.000604	34	82
CL232	Actinobacteria	0.22	0.23±0.20 a	0.07±0.08 a	0.12±0.27 a	0.02±0.05 a	0.94±0.42 a	0.25±0.11 a	0.04±0.03 a	0.07±0.04 a	AEU36797.1|Beta-glucosidase [Granulicella mallensis MP5ACTX8]	2.44E-21	61.7	98.4
CL240	Actinobacteria	0.23	0.0±0.0 a	0.29±0.6 ab	0.39±0.81 ab	0.76±1.87 ab	0.23±0.40 ab	0.05±0.07 a	0.06±0.07 a	0.12±0.15 a	AGL19386.1|beta-glucosidase-like glycosyl hydrolase [Actinoplanes sp. N902-109]	0.000285	54.2	54.5
CL271	Actinobacteria	0.23	0.02±0.03 a	0.19±0.43 a	0.25±0.61 a	0.0±0.0 a	1.03±1.21 ab	0.31±0.42 a	0.02±0.03 a	0.0±0.0 a	AGA90825.1|beta-glucosidase-like glycosyl hydrolase [Thioflavicoccus mobilis 8321]	0.000888	46.4	52.8
CL270	GammaProteoBacteria	0.20	0.0±0.0 b	0.0±0.0 b	0.30±0.73 a	1.09±2.66 a	0.0±0.0 a b	0.0±0.0 b	0.16±0.07 b	0.09±0.04 b	AJE39263.1|beta-glucosidase [Streptomyces nodosus]	0.003439	44.4	37.5
CL280	GammaProteoBacteria	0.21	0.5±1.19 b	0.048±0.08 a	0.41±0.50 b	0.12±0.16 b	0.30±0.29 b	0.20±0.15 a	0.06±0.05 a	0.07±0.07 a	AIF46589.1|beta-glucosidase [Dyella japonica A8]	2.35E-44	64.7	100
CL286	Fermicutes	0.17	1.05±2.2 a	0.33±0.8 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	AGA57676.1|beta-glucosidase-like glycosyl hydrolase [Thermobacillus composti KWC4]	0.005729	46.9	66.7
CL314	Actinobacteria	0.16	0.01±0.02 a	0.35±0.80 a	0.16±0.35 a	0.73±1.78 b	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	0.01±0.02 a	CCE74959.1|beta-glycosidase, glycosyl hydrolase family 3 [Clavibacter michiganensis subsp. nebraskens	0.003575	44	71.4
CL316	Actinobacteria	0.07	0.0±0.0 a	0.01±0.012 a	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	0.02±0.03 a	0.36±0.20 a	0.17±0.09 a	AEU36950.1|Beta-glucosidase [Granulicella mallensis MP5ACTX8]	2.70E-53	90.7	100
CL325	Actinobacteria	0.18	0.67±1.6 a	0.13±0.21 a	0.20±0.45 a	0.01±0.028 a	0.08±0.14 a	0.02±0.03 a	0.02±0.05 a	0.3±0.73 a	AAU93797.1|beta-glucosidase [Aeromicrobium erythreum]	0.000253	65	43.5
CL340	Fermicutes	0.29	0.0±0.0 c	0.0±0.0 c	0.74±1.07 a	1.40±3.41 b	0.039±0.05 c	0.03±0.02 c	0.11±0.07 c	0.03±0.03 c	ADB10263.1|bgl2 Beta-glucosidase [Bifidobacterium dentium Bd1]	0.004163	38.2	69.4
CL349	AlphaProteoBacteria	0.18	0.22±0.43 a	0.02±0.06 a	0.55±0.94 a	0.0±0.0 a	0.21±0.25 a	0.23±0.31 a	0.14±0.22 a	0.07±0.06 a	CCA90080.1|beta-glucosidase [Novosphingobium sp. PP1Y]	0.000729	54.8	46.3
CL353	BetaProteoBacteria	0.16	0.40±0.76 a	0.09±0.15 a	0.01±0.01 a	0.0±0.0 a	0.31±0.34 a	0.38±0.44 a	0.08±0.05 a	0.05±0.04 a	ADJ66244.1|periplasmic beta-D-glucoside glucohydrolase, protein [Herbaspirillum seropedicae SmR1]	0.000992	37	41.5
CL360	GammaProteoBacteria	0.27	0.01±0.02 a	1.44±3.52 a	0.25±0.62 a	0.01±0.03 a	0.30±0.20 a	0.08±0.09 a	0.03±0.02 a	0.02±0.02 a	AJB75233.1|beta-D-glucoside glucohydrolase [Klebsiella pneumoniae]	0.000526	53.3	71.4
CL373	Actinobacteria	0.17	0.69±1.53 aa	0.08±0.15 a	0.022±0.05 a	0.08±0.16 a	0.19±0.30 a	0.3±0.5 a	0.01±0.02 a	0.02±0.03 a	ACO33751.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	5.62E-55	79.4	100
CL389	Cyanobacteria	0.10	0.0±0.0 a	0.03±0.04 a	0.0±0.0 a	0.0±0.0 a	0.03±0.038 a	0.47±0.44 a	0.03±0.02 a	0.21±0.16 a	CAK24645.1|Beta-glycosidase of family GH3; possible N-acetyl b-glucosaminidase [Synechococcus sp. W	0.002282	50	43.2
CL426	Actinobacteria	0.10	0.04±0.06 a	0.035±0.045 a	0.45±0.40 a	0.0±0.0 a	0.03±0.07 a	0.03±0.05 a	0.18±0.1 a	0.06±0.04 a	AHJ97420.1|beta-glucosidase [Hymenobacter swuensis DY53]	6.92E-05	64.3	34.1
CL425	Bacteriodetes	0.10	0.01±0.02 b	0.0±0.0 b	0.63±1.53 a	0.01±0.03 b	0.0±0.0 b	0.0±0.0 b	0.05±0.07 b	0.06±0.08 b	CBL55785.1|Beta-glucosidase [Propionibacterium freudenreichii subsp. shermanii CIRM-BIA1]	2.52E-05	48.3	45.3
CL427	GammaProteoBacteria	0.11	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.1±0.13 a	0.77±1.74 b	0.01±0.02 a	0.01±0.02 a	NP_637141.1|glucan 1,4-beta-glucosidase [Xanthomonas campestris pv. campestris str. ATCC 33913]	0.008185	50	47.1
CL428	Fermicutes	0.08	0.0±0.0 b	0.6±1.6 a	0.01±0.02 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0b	0.0±0.0 b	AGA57760.1|beta-glucosidase-like glycosyl hydrolase [Thermobacillus composti KWC4]	0.003556	48.6	63.8
CL497	Acidobacteria	0.10	0.1±0.21 a	0.0±0.0 a	0.01±0.01 a	0.07±0.16 a	0.41±0.27 a	0.13±0.07 a	0.05±0.10 a	0.05±0.06 a	ACO32765.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	1.71E-51	77.5	100
CL501	Actinobacteria	0.17	0.01±0.03 a	0.0±0.0 a	0.44±1.07 b	0.85±2.07 b	0.0±0.0 a	0.0±0.0 a	0.05±0.0 a	0.01±0.01 a	AGT84960.1|beta-glucosidase [Amycolatopsis mediterranei RB]	0.001887	44.8	78.4
CL504	Actinobacteria	0.10	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.39±0.33 b	0.38±0.24 b	0.01±0.01 a	0.04±0.07 a	BAJ74999.1|beta-glucosidase-related glycosidase [Microbacterium testaceum StLB037]	0.001631	51.4	35.6
CL519	Actinobacteria	0.08	0.42±0.71 b	0.16±0.34 a	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	0.01±0.02 a	0.04±0.05 a	0.03±0.04 a	ACU84041.1|beta-glucosidase-like glycosyl hydrolase [Brachybacterium faecium DSM 4810]	0.006599	52	59.5
CL549	Actinobacteria	0.16	0.33±0.76 a	0.05±0.11 b	0.11±0.26 b	0.77±1.87 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	ADX74131.1|beta-glucosidase-like glycosyl hydrolase [Arthrobacter phenanthrenivorans Sphe3]	0.000732	44.8	85.3
CL557	Cyanobacteria	0.07	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.02±0.03 a	0.40±0.39 a	0.02±0.02 a	0.12±0.12 a	CAK24645.1|Beta-glycosidase of family GH3; possible N-acetyl b-glucosaminidase [Synechococcus sp. W	0.004307	50	43.2
CL570	Unknown	0.09	0.36±0.73 a	0.033±0.06 a	0.14±0.32 a	0.0±0.0 a	0.03±0.03 a	0.06±0.13 a	0.04±0.07 a	0.04±0.09 a	AHB24361.1|beta-glucosidase 1-6, partial [uncultured bacterium]	0.001306	34	93
CL575	Actinobacteria	0.13	0.18±0.40 a	0.038±0.062 a	0.39±0.28 b	0.42±0.96 b	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ACU84041.1|beta-glucosidase-like glycosyl hydrolase [Brachybacterium faecium DSM 4810]	0.001698	34.9	68.3
CL581	Actinobacteria	0.07	0.33±0.80 a	0.05±0.12 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.09±0.04 a	0.06±0.05 a	CBK71422.1|Beta-glucosidase-related glycosidases [Bifidobacterium pseudocatenulatum D2CA]	0.009253	41.4	80.6
CL612	Bacteriodetes	0.10	0.13±0.27 a	0.02±0.04 a	0.56±1.37 a	0.0±0.0 a	0.09±0.13 a	0.03±0.03 a	0.0±0.0 a	0.0±0.0 a	AGU13698.1|putative beta-glucosidase Bgl2 [Bacteroides uniformis]	0.008798	41.4	51.8
CL645	Acidobacteria	0.07	0.02±0.05 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.32±0.30 a	0.14±0.13 a	0.05±0.03 a	0.04±0.03 a	ACO32765.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	1.51E-41	75.5	100
CL647	Actinobacteria	0.08	0.01±0.02 a	0.01±0.02 a	0.01±0.02 a	0.03±0.05 a	0.31±0.14 a	0.18±0.13 a	0.01±0.01 a	0.04±0.08 a	CEA09596.1|Periplasmic beta-glucosidase precursor [Arthrobacter sp. 11W110_air]	0.006477	41.7	64.9
CL664	GammaProteoBacteria	0.05	0.0±0.0 a	0.40±0.98 a	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	AIX50662.1|beta-D-glucoside glucohydrolase [Pantoea sp. PSNIH1]	0.00436	57.1	32.6
CL677	Actinobacteria	0.10	0.02±0.05 b	0.69±1.68 a	0.032±0.08 b	0.0±0.0 b	0.0±0.0 b	0.01±0.02 b	0.03±0.02 b	0.03±0.03 b	AGK76940.1|Beta-glucosidase [Streptomyces fulvissimus DSM 40593]	0.006404	35.4	85.7
CL740	Acidobacteria	0.04	0.0±0.0 a	0.0±0.0 a	0.09±0.15 a	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	0.16±0.15 a	0.06±0.05 a	ACO33751.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	9.04E-53	76.5	100
CL742	Firmicutes	0.13	0.0±0.0 a	0.0±0.0 a	0.25±0.62 a	0.76±1.90 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CBL14295.1|Beta-glucosidase-related glycosidases [Roseburia intestinalis XB6B4]	0.007856	58.8	34.7
CL756	Actinobacteria	0.10	0.32±0.22 b	0.30±0.36 b	0.05±0.09 ab	0.01±0.01 ab	0.06±0.07 ab	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	CAJ89978.1|putative beta-glucosidase [Streptomyces ambofaciens ATCC 23877]	4.75E-06	53.8	45.6
CL763	GammaProteoBacteria	0.12	0.0±0.0 a	0.0±0.0 a	0.2±0.27 ab	0.79±0.80 ab	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	ACF52074.1|Beta-glucosidase [Stenotrophomonas maltophilia R551-3]	0.000727	44.1	58.6
CL800	Actinobacteria	0.09	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.02±0.03 a	0.67±1.54 b	0.0±0.0 a	0.0±0.0 a	ACZ20198.1|beta-glucosidase-like glycosyl hydrolase [Sanguibacter keddieii DSM 10542]	0.000162	47.8	43.4
CL814	BetaProteoBacteria	0.09	0.0±0.0 b	0.70±1.71 a	0.0±0.0 b	0.0±0.0b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	ADJ66244.1|periplasmic beta-D-glucoside glucohydrolase, protein [Herbaspirillum seropedicae SmR1]	0.008107	47.4	43.2
CL821	Actinobacteria	0.08	0.0±0.0 a	0.54±1.31 a	0.01±0.03 a	0.02±0.05 a	0.0±0.0 a	0.0±0.0 a	0.03±0.02 a	0.01±0.02 a	ACO33751.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	2.35E-52	76.5	100
CL851	Actinobacteria	0.09	0.01±0.013 a	0.0±0.0 a	0.68±1.24 a	0.06±0.15 a	0.0±0.0 a	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	AGA57213.1|beta-glucosidase-like glycosyl hydrolase [Thermobacillus composti KWC4]	0.00787	41.2	69.4
CL887	Actinobacteria	0.09	0.0±0.0 a	0.0±0.0 a	0.50±0.78 a	0.21±0.52 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ACZ20198.1|beta-glucosidase-like glycosyl hydrolase [Sanguibacter keddieii DSM 10542]	0.004902	50	66.7
CL891	Actinobacteria	0.13	0.80±1.96 a	0.11±0.26 a	0.032±0.08 a	0.02±0.03 a	0.0±0.0 a	0.0±0.0 a	0.04±0.02 a	0.02±0.02 a	BAA31467.1|beta-glucosidase [Komagataeibacter sucrofermentans DSM 15973]	0.001863	40.9	57.9
CL898	Actinobacteria	0.03	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.02±0.03 a	0.018±0.023 a	0.11±0.06 a	0.07±0.06 a	AJE81369.1|periplasmic beta-glucosidase [Streptomyces albus]	0.003815	45.7	50.7
CL905	BetaProteoBacteria	0.02	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.01±0.01 bc	0.01±0.013 bd	0.10±0.03 a	0.09±0.04 ab	AEA65019.1|Beta-glucosidase [Burkholderia gladioli BSR3]	0.003304	50	26.5
CL907	Actinobacteria	0.06	0.42±0.85 a	0.03±0.06 a	0.01±0.02 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ACZ20341.1|beta-glucosidase-like glycosyl hydrolase [Sanguibacter keddieii DSM 10542]	0.007683	42.9	56
CL925	AlphaProteoBacteria	0.07	0.02±0.06 a	0.50±1.23 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	NP_420857.1|1,4-beta-D-glucan glucohydrolase D [Caulobacter crescentus CB15]	0.002887	48.5	73.3
CL930	Actinobacteria	0.06	0.0±0.0 a	0.40±0.96 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.04±0.04 a	0.01±0.02 a	CCA60020.1|Beta-glucosidase [Streptomyces venezuelae ATCC 10712]	0.000429	50	68.2
CL1003	GammaProteoBacteria	0.05	0.02±0.03 a	0.32±0.42 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.01±0.02 a	0.03±0.02 a	0.03±0.03 a	AHE61702.1|beta-glucosidase, periplasmic [Escherichia albertii KF1]	0.004902	51.9	75
CL1001	GammaProteoBacteria	0.22	0.0±0.0 a	0.0±0.0 a	0.26±0.62 a	1.50±3.67 b	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CAW28330.1|periplasmic beta-glucosidase [Pseudomonas aeruginosa LESB58]	0.002594	48.3	76.3
CL1039	Actinobacteria	0.05	0.0±0.0 b	0.0±0.0 b	0.40±0.98 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	ACZ20198.1|beta-glucosidase-like glycosyl hydrolase [Sanguibacter keddieii DSM 10542]	0.00787	45.5	68.8
CL1070	Acidobacteria	0.02	0.0±0.0 a	0.016±0.039 a	0.0±0.0 a	0.01±0.02 a	0.02±0.03 a	0.0±0.0 a	0.08±0.06 a	0.05±0.11 a	ACO32765.1|beta-glucosidase [Acidobacterium capsulatum ATCC 51196]	8.55E-39	58.8	100
CL1086	Actinobacteria	0.10	0.0±0.0 a	0.0±0.0 a	0.22±0.19 a	0.56±0.75 a	0.0±0.0 a	0.01±0.02 a	0.03±0.04 a	0.0±0.0 a	AJE81269.1|beta-glucosidase [Streptomyces albus]	0.000494	50	37.4
CL1135	Actinobacteria	0.02	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.17±0.3 a	0.01±0.02 a	AFR08388.1|thermostable beta-glucosidase B [Nocardiopsis alba ATCC BAA-2165]	0.00028	37.8	92.5
CL1218	AlphaProteoBacteria	0.11	0.71±1.71 a	0.06±0.19 a	0.01±0.01 a	0.048±0.12 a	0.02±0.02 a	0.02±0.02 a	0.0±0.0 a	0.0±0.0 a	ABZ71268.1|Beta-glucosidase [Caulobacter sp. K31]	0.000727	50	82.1
CL1245	AlphaProteoBacteria	0.08	0.0±0.0 a	0.0±0.0 a	0.12±0.42 a	0.45±0.93 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ADG12157.1|Beta-glucosidase [Caulobacter segnis ATCC 21756]	0.003404	42.9	41.2
CL1259	Actinobacteria	0.08	0.6±1.47 a	0.037±0.09 a	0.01±0.02 a	0.02±0.05 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	BAK33792.1|beta-glucosidase [Microlunatus phosphovorus NM-1]	0.002828	50	34.8
CL1285	AlphaProteoBacteria	0.06	0.0±0.0 a	0.0±0.0 a	0.50±1.21 a	0.02±0.03 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	NP_419921.1|beta-glucosidase [Caulobacter crescentus CB15]	0.007856	36.4	67.3
CL1314	Actinobacteria	0.35	0.0±0.0 a	0.0±0.0 a	0.07±0.20 a	2.74±6.72 a	0.0±0.0 a	0.0±0.0 a	0.01±0.02 a	0.01±0.01 a	ACZ66247.1|beta-glycosidase [Terrabacter sp. Gsoil 3082]	0.003052	57.9	55.9
CL1873	Actinobacteria	0.05	0.0±0.0 a	0.0±0.0 a	0.04±0.1 a	0.34±0.84 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AGT85851.1|beta-glucosidase [Amycolatopsis mediterranei RB]	0.000113	47.1	65.4
CL1890	Actinobacteria	0.09	0.0±0.0 a	0.01±0.01 a	0.0±0.0 a	0.55±1.30 a	0.12±0.2 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AGS69321.1|beta-glucosidase [Streptomyces collinus Tu 365]	0.004349	53.6	71.8
CL1893	Actinobacteria	0.05	0.0±0.0 a	0.32±0.78 a	0.0±0.0 a	0.0±0.0 a	0.05±0.06 a	0.02±0.02 a	0.0±0.0 a	0.0±0.0 a	AGM07442.1|beta-glucosidase [Amycolatopsis orientalis HCCB10007]	0.004407	55.6	60
CL1916	Actinobacteria	0.21	0.0±0.0 a	0.0±0.0 a	0.11±0.2 a	1.53±3.77 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ACZ20402.1|beta-glucosidase-like glycosyl hydrolase [Sanguibacter keddieii DSM 10542]	0.005123	40	67.3
CL1977	Actinobacteria	0.04	0.0±0.0 a	0.0±0.0 a	0.30±0.57 a	0.01±0.028 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AER06394.1|glycoside hydrolase family 3 protein [Propionibacterium acnes ATCC 11828]	0.007499	52.2	53.5
CL2080	GammaProteoBacteria	0.04	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.34±0.83 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AGA75162.1|beta-D-glucoside glucohydrolase [Pseudomonas putida HB3267]	0.005458	52	58.1
CL2071	GammaProteoBacteria	0.06	0.028±0.07 a	0.01±0.02a	0.07±0.1 a	0.33±0.64 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEO43250.1|Beta-glucosidase-related glycosidase [Xanthomonas axonopodis pv. citrumelo F1]	0.000546	36.4	55.7
CL2383	AlphaProteoBacteria	0.05	0.34±0.83 a	0.021±0.05 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ABZ71531.1|Beta-glucosidase [Caulobacter sp. K31]	0.002217	42.9	51.2
CL2556	Actinobacteria	0.18	0.0±0.0 a	0.0±0.0 a	0.03±0.06 a	1.40±3.44 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AGT88123.1|beta-glucosidase [Amycolatopsis mediterranei RB]	0.00216	55.6	48.6
CL2627	Actinobacteria	0.13	0.0±0.0 a	0.0±0.0 a	0.06±0.15 a	1.01±2.45 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCH88398.1|beta-glucosidase (Glycosyl hydrolase,family3) [Modestobacter marinus]	0.009981	42.3	54.2

Supplementary Table 6: Overview of taxonomical assignment of most abundant OTUs of fungal β-glucosidase genes from GH3 family, their abundance and districution among L and H horizons and among DNA and RNA in summer and winter. Abundance data are expressed as means (%) and standard deviations from six different sites. Statastically significant differences in relative abundance among HLR, HZR, LLR, LZR, HLD, HZD, LLD and LZD (ANOVA followed by TuKey Post-hoc) are indicated by different letters.

OTUs	Assignment	Mean Abundance (%)	HLR (%)	HZR (%)	LLR (%)	LZR (%)	HLD (%)	HZD (%)	LLD (%)	LZD (%)	Best Hit	E value	Similarity (%)	Coverage (%)
CL0	Basidiomycota	13.35	20.53±32.90 bc	0.1±0.24 c	30.9±18.06 ab	55.26±17.89 a	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	AFK65507.1|beta glucosidase [Glaciozyma antarctica]	8.67E-40	55.7	100
CL1	Ascomycota	9.60	4.9±5.80 b	7.37±11.53 b	1.4±2.48 b	0.13±0.17 b	24.67±6.81 a	24.98±3.73 a	6.77±5.14 b	6.57±2.34 b	AGW24289.1|beta-glucosidase [Penicillium oxalicum]	1.02E-59	90.5	100
CL2	Ascomycota	6.29	4.27±5.12 bc	6.58±7.47 abc	0.36±0.72 c	0.32±0.53 c	10.7±6.31 ab	13.18±2.75 a	7.35±4.66 abc	7.55±4.70 abc	CCD50179.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	8.62E-39	80	42.1
CL3	Ascomycota	8.02	9.4±5.37 b	6.52±6.13 b	2.28±3.19 b	1.2±1.45 b	18.7±8.38 a	18.68±4.21 a	3.05±2.66 b	4.33±2.78 b	CBX93055.1|similar to beta-glucosidase [Leptosphaeria maculans JN3]	4.34E-53	83.3	100
CL4	Ascomycota	5.22	3.4±5.73 b	0.97±2.37 b	6.28±2.59 ab	2.4±2.40 b	3.23±3.95 b	1.13±1.22 b	16.87±14.87 a	7.5±4.04 ab	AFU51372.1|beta-glucosidase [Thermoascus aurantiacus]	1.40E-51	81.9	98.9
CL5	Ascomycota	3.94	0.78±1.22 b	5.2±10.16 ab	0.56±0.28 b	0.55±0.55 b	1.13±0.99 b	0.63±0.37 b	10.18±4.32 a	12.48±1.88 a	AAP57760.1|Cel3e [Trichoderma reesei]	2.03E-28	69.8	74.1
CL6	Ascomycota	3.46	1.18±1.91 bc	5.97±7.1 abc	0.25±0.33 c	0.65±1.10 c	1.1±0.62 c	0.87±0.37 c	7.28±2.84 ab	10.36±3.27 a	CEF72029.1|unnamed protein product [Fusarium graminearum]	1.22E-53	83.3	100
CL7	Ascomycota	2.59	0.78±1.92 a	8.98±13.65 a	0.0±0.0 a	0.98±2.31 a	2.23±2.26 a	4.0±1.75 a	2.02±1.27 a	1.73±1.40 a	CCT66374.1|probable beta-glucosidase [Fusarium fujikuroi IMI 58289]	2.45E-54	83.2	100
CL10	Ascomycota	2.63	1.33±2.07 a	1.0±2.08 a	0.15±0.27 a	0.03±0.08 a	5.76±7.01 a	5.75±4.43 a	3.02±4.37 a	3.98±2.65 a	CEF72029.1|unnamed protein product [Fusarium graminearum]	1.22E-53	83.3	100
CL11	Ascomycota	2.34	5.53±7.54 a	0.017±0.04 a	1.23±1.56 a	0.57±0.48 a	0.41±0.49 a	0.3±0.21 a	5.1±2.63 a	5.58±3.37 a	AAP57760.1|Cel3e [Trichoderma reesei]	3.92E-28	69.8	74.1
CL13	Ascomycota	2.55	6.41±12.27 a	0.0±0.0 a	9.1±5.10 a	4.85±6.88 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.52E-56	85.3	100
CL14	Ascomycota	1.54	0.77±1.88 b	0.0±0.0 b	0.25±0.35 b	0.3±0.73 b	0.23±0.2 b	0.03±0.05 b	6.4±6.49 a	4.3±2.64 ab	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	5.09E-52	82.8	56.6
CL16	Ascomycota	2.22	0.0±0.0 a	7.87±19.22 a	1.41±2.70 a	8.5±7.12 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	8.72E-46	77.9	100
CL17	Ascomycota	2.40	5.72±13.90 a	0.8±1.52 a	0.0±0.0 a	0.0±0.0 a	9.35±11.55 a	1.83±2.53 a	1.17±2.80 a	0.3±0.69 a	AFU51372.1|beta-glucosidase [Thermoascus aurantiacus]	1.68E-49	78.7	98.9
CL18	Ascomycota	0.79	0.0±0.0 a	3.92±9.54 a	0.1±0.11 a	0.06±0.08 a	0.06±0.16 a	0.12±0.11 a	0.9±0.80 a	1.17±0.44 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.65E-41	88.4	100
CL20	Ascomycota	1.38	1.7±4.16 b	0.0±0.0 b	9.35±10.87 a	0±0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	EAL91070.1|beta-glucosidase, putative [Aspergillus fumigatus Af293]	4.09E-30	56.8	100
CL22	Ascomycota	1.38	2.77±4.32 a	4.4±7.57 a	0.48±1.18 a	0.05±0.08 a	0.17±0.16 a	0.18±0.19 a	1.07±1.57 a	1.93±1.29 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.65E-59	87.4	100
CL23	Ascomycota	1.12	1.55±3.28 a	0.0±0.0 a	4.21±4.64 a	3.17±4.74 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	EAL91070.1|beta-glucosidase, putative [Aspergillus fumigatus Af293]	2.06E-28	53.4	100
CL24	Ascomycota	0.83	0.0±0.0 c	0.0±0.0 c	0.63±0.98 bc	0.08±0.20 c	0.15±0.19 c	0.1±0.09 c	2.93±1.81 a	2.73±2.71 ab	AAP57760.1|Cel3e [Trichoderma reesei]	9.04E-28	68.3	74.1
CL25	Ascomycota	1.07	0.95±2.33 a	0.93±2.29 a	0.02±0.04 a	0.0±0.0 a	1.92±0.80 a	1.65±0.97 a	1.82±1.31 a	1.27±0.94 a	AAP57755.1|Cel3b [Trichoderma reesei]	1.30E-58	91.5	98.9
CL27	Ascomycota	1.10	0.1±0.2 a	3.78±6.59 a	0.37±0.43 a	0.15±0.36 a	0.62±0.36 a	2.08±2.08 a	0.57±1.18 a	1.15±1.02 a	AEL79685.1|GH3 beta-glucosidase [Aspergillus saccharolyticus]	6.35E-54	83.2	100
CL28	Ascomycota	0.49	0.0±0.0 a	2.52±6.16 a	0.03±0.08 a	0.0±0.0 a	0.52±1.12 a	0.68±0.9 a	0.05±0.12 a	0.17±0.28 a	AFU51372.1|beta-glucosidase [Thermoascus aurantiacus]	3.94E-48	76.6	98.9
CL30	Ascomycota	0.70	0.1±0.24 a	1.87±4.57 a	0.017±0.04 a	0.0±0.0 a	0.03±0.05 a	0.03±0.08 a	1.42±1.20 a	2.12±1.35 a	AFH41575.1|beta-glucosidase [Periconia sp. BCC 2871]	8.34E-35	74.2	26.3
CL31	Ascomycota	1.34	1.08±2.60 a	2.88±7.06 a	0.0±0.0 a	0.0±0.0 a	2.63±1.32 a	3.77±2.79 a	0.17±0.08 a	0.22±0.15 a	AGW24289.1|beta-glucosidase [Penicillium oxalicum]	6.34E-62	94.7	100
CL33	Basidiomycota	0.69	0.0±0.0 a	0.0±0.0 a	1.83±3.19 a	3.65±5.28 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	BAB85988.1|beta-glucosidase, partial [Phanerochaete chrysosporium]	8.72E-42	68.4	100
CL34	Ascomycota	0.65	0.0±0.0 a	4.43±9.04 a	0.0±0.0 a	0.02±0.04 a	0.07±0.12 a	0.53±0.58 a	0.08±0.07 a	0.1±0.15 a	CEF78182.1|unnamed protein product [Fusarium graminearum]	2.03E-43	73.9	100
CL35	Ascomycota	0.38	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.4±0.98 b	0.01±0.04 b	0.03±0.05 b	0.93±1.17 ab	1.67±1.15 a	CEF72029.1|unnamed protein product [Fusarium graminearum]	9.49E-54	82.3	100
CL36	Ascomycota	0.45	0.38±0.67 a	0.37±0.9 a	0.016±0.04 a	0.0±0.0 a	0.03±0.05 a	0.07±0.16 a	1.25±2.28 a	1.45±0.77 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.16E-40	73.9	33.3
CL39	Ascomycota	0.36	0.02±0.04 a	0.0±0.0 a	0.87±1.52 a	1.97±4.62 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AAL69548.3|beta-glucosidase [Rasamsonia emersonii]	1.48E-48	75.8	100
CL40	Ascomycota	0.45	0.0±0.0 a	0.97±2.37 a	0.0±0.0 a	0.35±0.86 a	0.17±0.22 a	0.95±1.63 a	0.23±0.57 a	0.92±2.10 a	AAP57755.1|Cel3b [Trichoderma reesei]	2.87E-48	86.2	98.9
CL41	Ascomycota	0.30	0.0±0.0 a	0.0±0.0 a	0.27±0.56 a	0.0±0.0 a	0.03±0.08 a	0.07±0.10 a	1.08±2.07 a	0.97±0.89 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.65E-59	87.4	100
CL42	Ascomycota	0.38	0.33±0.82 a	0.0±0.0 a	2.62±4.70 a	0.12±0.24 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	XP_003719616.1|beta-glucosidase 2 [Magnaporthe oryzae 70-15]	5.28E-41	70.8	100
CL45	Ascomycota	0.36	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.03±0.08 a	0.17±0.41 a	0.02±0.04 a	1.88±3.47 a	0.81±1.56 a	CEF78744.1|unnamed protein product [Fusarium graminearum]	2.12E-42	75.9	100
CL46	Ascomycota	0.90	0.03±0.08 a	5.5±10.57 a	0.07±0.16 a	0.0±0.0 a	0.67±0.45 a	0.83±0.41 a	0.0±0.0 a	0.06±0.12 a	CEF83026.1|unnamed protein product [Fusarium graminearum]	8.05E-53	78.9	100
CL47	Ascomycota	0.48	1.75±4.27 a	0.0±0.0 a	0.05±0.12 a	0.0±0.0 a	0.37±0.29 a	0.4±0.22 a	0.77±0.48 a	0.47±0.14 a	AAP57760.1|Cel3e [Trichoderma reesei]	3.16E-47	81.6	100
CL49	Ascomycota	0.26	0.0±0.0 b	0.0±0.0 b	0.03±0.08 b	0.02±0.04 b	0.07±0.08 b	0.0±0.0 b	0.72±0.60 ab	1.23±1.24 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.93E-47	85.3	100
CL50	Ascomycota	0.34	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.53±1.07 a	2.15±4.70 a	0.0±0.0 a	0.0±0.0 a	CAA26662.1|unnamed protein product [Wickerhamomyces anomalus]	1.19E-25	66	56.4
CL51	Ascomycota	0.44	0.0±0.0 a	2.18±5.35 a	0.0±0.0 a	0.0±0.0 a	0.7±1.10 a	0.63±0.76 a	0.0±0.0 a	0.02±0.04 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	6.34E-58	88.4	100
CL52	Ascomycota	0.41	0.13±0.33 a	2.1±5.01 a	0.0±0.0 a	0.0±0.0 a	0.52±1.03 a	0.47±0.46 a	0.05±0.08 a	0.07±0.10 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	3.26E-57	88.4	100
CL54	Ascomycota	0.24	0.0±0.0 a	0.0±0.0 a	0.02±0.04 a	0.02±0.04 a	0.03±0.05 a	0.03±0.05 a	0.35±0.76 a	1.48±2.41 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.07E-60	91.6	100
CL55	Ascomycota	0.23	0.0±0.0 c	0.0±0.0 c	0.17±0.28 bc	0.13±0.32 bc	0.0±0.0 c	0.02±0.04 c	0.9±0.56 a	0.58±0.33 ab	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.87E-52	78.9	100
CL56	Ascomycota	0.44	0.92±1.62 a	1.97±3.68 a	0.3±0.13 a	0.37±0.30 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	EAL91070.1|beta-glucosidase, putative [Aspergillus fumigatus Af293]	6.40E-28	53.4	100
CL59	Basidiomycota	0.26	0.017±0.04 a	0.0±0.0 a	1.9±4.29 a	0.15±0.37 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AAC26489.1|cellulose-binding beta-glucosidase [Phanerochaete chrysosporium]	6.48E-41	67.4	100
CL61	Ascomycota	0.43	1.78±4.37 a	1.57±3.84 a	0.0±0.0 a	0.0±0.0 a	0.03±0.08 a	0.02±0.04 a	0.03±0.05 a	0.0±0.0 a	CAK46489.1|unnamed protein product [Aspergillus niger]	1.48E-57	95.4	97.8
CL62	Ascomycota	0.31	1.37±1.83 a	0.0±0.0 a	0.47±0.90 a	0.68±1.01 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD50179.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	3.40E-44	75.8	100
CL63	Ascomycota	0.29	0.3±0.55 a	0.0±0.0 a	0.38±0.62 a	0.12±0.14 a	0.57±0.41 a	0.65±0.43 a	0.08±0.07 a	0.25±0.23 a	CBX93055.1|similar to beta-glucosidase [Leptosphaeria maculans JN3]	3.42E-54	83.3	100
CL65	Ascomycota	0.33	1±1.78 a	1.35±3.31 a	0.1±0.2 a	0.0±0.0 a	0.06±0.16 a	0.0±0.0 a	0.017±0.04 a	0.07±0.08 a	ABP88968.1|beta-glucosidase [Penicillium brasilianum]	8.72E-50	78.7	98.9
CL66	Ascomycota	0.16	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.01±0.04 b	0.0±0.0 b	0.57±0.61 a	0.7±0.49 a	CCD47324.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.89E-42	70.7	51.8
CL67	Ascomycota	0.34	0.18±0.45 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	1.63±2.6 a	0.82±1.29 a	0.0±0.0 a	0.08±0.20 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	5.00E-60	91.6	100
CL68	Ascomycota	0.22	0.0±0.0 a	0.0±0.0 a	1.55±3.36 a	0.03±0.08 a	0.0±0.0 a	0.0±0.0 a	0.1±0.09 a	0.07±0.08 a	AEL79685.1|GH3 beta-glucosidase [Aspergillus saccharolyticus]	4.27E-53	82.1	100
CL69	Ascomycota	0.19	0.08±0.20 b	0.0±0.0 b	1.32±1.41 a	0.15±0.32 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	AER93027.1|beta-glucosidase, partial [Aspergillus aculeatus]	8.69E-41	68.4	100
CL70	Ascomycota	0.31	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.1±0.24 a	0.45±1.10 a	1.65±3.85 a	0.05±0.12 a	0.23±0.52 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.09E-56	85.3	100
CL71	Ascomycota	0.20	0.0±0.0 b	0.0±0.0 b	1.55±1.98 a	0.07±0.12 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	AER93027.1|beta-glucosidase, partial [Aspergillus aculeatus]	2.07E-40	69.5	100
CL72	Ascomycota	0.16	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.12±0.14 b	0.02±0.04 b	0.58±0.35 a	0.58±0.59 a	EAA57725.1|hypothetical protein AN5976.2 [Aspergillus nidulans FGSC A4]	7.74E-46	69.7	100
CL74	Ascomycota	0.17	0.1±0.24 b	0.0±0.0 ab	0.12±0.18 ab	0.13±0.24 ab	0.2±0.39 ab	0.0±0.0 b	0.53±0.46 a	0.27±0.24 ab	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	3.64E-47	85.3	100
CL75	Ascomycota	0.20	0.0±0.0 a	0.0±0.0 a	1.05±1.36 a	0.53±1.07 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AER93027.1|beta-glucosidase, partial [Aspergillus aculeatus]	2.07E-40	69.5	100
CL77	Basidiomycota	0.19	0.07±0.10 b	0.0±0.0 b	0.5±0.53 ab	0.97±0.74 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	AFK65507.1|beta glucosidase [Glaciozyma antarctica]	3.09E-39	55.7	100
CL79	Ascomycota	0.18	0.08±0.13 a	0.0±0.0 a	0.57±0.36 a	0.58±1.01 a	0.0±0.0 a	0.0±0.0 a	0.08±0.07 a	0.08±0.12 a	AFC40275.1|beta-glucosidase family 3 protein [Stachybotrys microspora]	5.41E-52	78.9	100
CL81	Ascomycota	0.16	0.07±0.10 b	0.0±0.0 b	1.22±0.94 a	0.017±0.04 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	AEL79685.1|GH3 beta-glucosidase [Aspergillus saccharolyticus]	4.74E-30	69	65.9
CL82	Ascomycota	0.19	0.03±0.082 b	0.02±0.04 b	0.0±0.0 b	0.0±0.0 b	0.57±0.35 a	0.43±0.29 a	0.25±0.18 ab	0.25±0.21 ab	CEF78744.1|unnamed protein product [Fusarium graminearum]	1.03E-30	71.4	62.2
CL85	Ascomycota	0.17	0.0±0.0 a	0.0±0.0 a	0.2±0.49 a	0.0±0.0 a	0.5±0.42 a	0.62±0.99 a	0.03±0.05 a	0.03±0.05 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	4.62E-46	89.5	100
CL87	Ascomycota	0.12	0.0±0.0 c	0.0±0.0 c	0.02±0.04 c	0.02±0.04 c	0.03±0.05 c	0.08±0.16 bc	0.36±0.30 ab	0.43±0.34 a	CBX93055.1|similar to beta-glucosidase [Leptosphaeria maculans JN3]	1.67E-53	82.3	100
CL89	Ascomycota	0.15	0.18±0.45 a	0.0±0.0 a	0.57±0.93 a	0.42±0.70 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AAF21242.1|beta-glucosidase precursor [Coccidioides posadasii]	4.27E-53	81.1	100
CL90	Ascomycota	0.30	1.55±3.79 a	0.02±0.04 a	0.0±0.0 a	0.0±0.0 a	0.35±0.31 a	0.12±0.16 a	0.15±0.23 a	0.18±0.15 a	CEF72029.1|unnamed protein product [Fusarium graminearum]	4.34E-53	82.3	100
CL91	Ascomycota	0.12	0.1±0.245 a	0.0±0.0 a	0.22±0.34 a	0.12±0.29 a	0.07±0.08 a	0.1±0.06 a	0.27±0.46 a	0.1±0.06 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.99E-58	88.4	100
CL92	Ascomycota	0.18	0.0±0.0 b	0.03±0.08 b	0.3±0.73 ab	0.0±0.0 b	0.72±0.52 a	0.35±0.42 ab	0.0±0.0 b	0.02±0.04 b	EAA63399.1|hypothetical protein AN2828.2 [Aspergillus nidulans FGSC A4]	4.13E-31	59	69.3
CL93	Ascomycota	0.16	0.0±0.0 a	0.02±0.04 a	0.0±0.0 a	0.0±0.0 a	0.42±0.29 a	0.58±0.79 a	0.2±0.29 a	0.06±0.08 a	AIB10099.1|glycoside hydrolase family 3 protein [Trichoderma koningiopsis]	1.30E-25	57.8	73.6
CL95	Ascomycota	0.12	0.0±0.0 a	0.0±0.0 a	0.38±0.94 a	0.57±1.38 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD47324.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.22E-47	74.5	100
CL96	Ascomycota	0.12	0.07±0.16 a	0.0±0.0 a	0.55±0.98 a	0.37±0.47 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD47324.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.22E-47	74.5	100
CL98	Ascomycota	0.11	0.13±0.33 a	0.0±0.0 a	0.17±0.40 a	0.3±0.53 a	0.13±0.13 a	0.1±0.13 a	0.02±0.04 a	0.03±0.05 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	3.64E-55	86.3	100
CL99	Ascomycota	0.12	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.98±2.36 a	0.0±0.0 a	0.0±0.0 a	CEF83026.1|unnamed protein product [Fusarium graminearum]	1.14E-47	79.2	70.6
CL101	Ascomycota	0.42	0.0±0.0 a	3.35±8.20 a	0.0±0.0a	0.0±0.0 a	0.0±0.0 a	0.017±0.04 a	0.0±0.0 a	0.02±0.04 a	AAP57760.1|Cel3e [Trichoderma reesei]	1.54E-50	86.2	100
CL103	Ascomycota	0.10	0.0±0.0 a	0.0±0.0 a	0.17±0.15 ab	0.0±0.0 a	0.03±0.05 a	0.02±0.04 a	0.25±0.18 ab	0.38±0.34 a	AAP57760.1|Cel3e [Trichoderma reesei]	2.28E-28	69.8	74.1
CL104	Ascomycota	0.09	0.0±0.0 a	0.0±0.0 a	0.017±0.04 a	0.0±0.0 a	0.02±0.04 a	0.0±0.0 a	0.28±0.45 a	0.43±0.82 a	AAP57760.1|Cel3e [Trichoderma reesei]	2.41E-28	69.8	74.1
CL108	Ascomycota	0.05	0.0±0.0 a	0.43±1.06 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AAP57755.1|Cel3b [Trichoderma reesei]	1.11E-48	88.3	98.9
CL110	Ascomycota	0.13	0.03±0.08 c	0.0±0.0 c	0.02±0.04 c	0.03±0.08 c	0.52±0.16 a	0.27±0.14 b	0.06±0.05 c	0.08±0.09 c	AAA34315.1|beta-glucosidase 2 precursor [Saccharomycopsis fibuligera]	2.25E-06	38.7	26.7
CL111	Ascomycota	0.09	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.47±0.53 a	0.22±0.14 ab	EAL84958.1|beta-glucosidase, putative [Aspergillus fumigatus Af293]	4.07E-20	70.2	45.2
CL112	Ascomycota	0.07	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.23±0.10 a	0.33±0.30 a	AAF21242.1|beta-glucosidase precursor [Coccidioides posadasii]	4.61E-48	76.2	75.7
CL115	Ascomycota	0.10	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.18±0.12 ab	0.58±0.70 a	0.0±0.0 b	0.05±0.08 b	CCD50179.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.42E-40	85	37.4
CL116	Basidiomycota	0.09	0.03±0.08 a	0.4±0.98 a	0.05±0.08 a	0.22±0.25 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CAE01320.1|beta glucosidase precursor [Uromyces viciae-fabae]	3.61E-42	59.6	100
CL119	Ascomycota	0.12	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.4±0.33 a	0.55±0.43 a	0.0±0.0 b	0.0±0.0 b	GAM40530.1|glycosyl hydrolase family 3 beta-glucosidase Bgl3A [Talaromyces cell	2.56E-34	72.4	45.7
CL121	Ascomycota	0.10	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.32±0.34 ab	0.43±0.46 a	0.02±0.04 b	0.03±0.08 b	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	3.11E-53	84.2	100
CL122	Ascomycota	0.11	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.38±0.54 a	0.32±0.44 a	0.1±0.15 a	0.08±0.07 a	AAP57755.1|Cel3b [Trichoderma reesei]	1.02E-59	94.7	98.9
CL124	Ascomycota	0.05	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.18±0.14 a	0.25±0.38 a	CCD52514.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	8.45E-51	88.7	55.9
CL125	Ascomycota	0.39	3.11±5.79 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEL79685.1|GH3 beta-glucosidase [Aspergillus saccharolyticus]	2.34E-41	69.5	100
CL126	Ascomycota	0.07	0.0±0.0 a	0.0±0.0 a	0.016±0.04 a	0.53±1.02 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CEF83026.1|unnamed protein product [Fusarium graminearum]	2.45E-50	76.8	100
CL128	Zygomycota	0.07	0.02±0.04 b	0.0±0.0 b	0.4±0.55 a	0.13±0.14 ab	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	BAO00904.1|beta glucosidase 1 [Mucor javanicus]	8.73E-38	66.3	100
CL129	Ascomycota	0.16	1.03±2.53 a	0.0±0.0 a	0.23±0.31 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AIB10099.1|glycoside hydrolase family 3 protein [Trichoderma koningiopsis]	2.26E-49	80	100
CL135	Ascomycota	0.29	2.27±5.55 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.03±0.05 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	9.96E-56	91.1	50.5
CL138	Ascomycota	0.06	0.0±0.0 b	0.0±0.0 b	0.02±0.04 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.27±0.22 a	0.17±0.15 ab	AER93027.1|beta-glucosidase, partial [Aspergillus aculeatus]	6.87E-51	80	100
CL141	Ascomycota	0.08	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.02±0.04 a	0.0±0.0 a	0.5±1.18 a	0.08±0.16 a	AFH41575.1|beta-glucosidase [Periconia sp. BCC 2871]	8.58E-29	63.3	60.6
CL142	Ascomycota	0.05	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.42±0.83 a	0.0±0.0 a	EAA63399.1|hypothetical protein AN2828.2 [Aspergillus nidulans FGSC A4]	2.06E-34	70.5	69.3
CL143	Ascomycota	0.07	0.0±0.0 a	0.18±0.45 a	0.0±0.0 a	0.0±0.0 a	0.03±0.05 a	0.31±0.59 a	0.0±0.0 a	0.0±0.0 a	CEF78744.1|unnamed protein product [Fusarium graminearum]	4.01E-30	59.8	100
CL144	Ascomycota	0.08	0.37±0.47 a	0.0±0.0 a	0.1±0.24 a	0.18±0.26 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD50179.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	3.40E-44	75.8	100
CL145	Ascomycota	0.12	0.63±1.05 a	0.0±0.0 a	0.2±0.23 a	0.1±0.13 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	ACD86466.1|beta-glucosidase [Penicillium decumbens]	5.62E-27	57.8	72.7
CL146	Zygomycota	0.17	1.38±3.39 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	EPB86303.1|hypothetical protein HMPREF1544_06945 [Mucor circinelloides f. circ	9.73E-42	72	53.2
CL150	Ascomycota	0.05	0.0±0.0 a	0.0±0.0 a	0.33±0.60 a	0.1±0.24 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CAK48253.1|unnamed protein product [Aspergillus niger]	6.40E-40	81.8	100
CL158	Ascomycota	0.06	0.12±0.27 a	0.08±0.20 a	0.0±0.0 a	0.0±0.0 a	0.02±0.04 a	0.28±0.51 a	0.0±0.0 a	0.017±0.04 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	3.86E-36	65.3	52.1
CL159	Ascomycota	0.07	0.017±0.04 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.25±0.21 a	0.27±0.40 a	0.0±0.0 a	0.017±0.04 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.30E-54	85.3	100
CL160	Basidiomycota	0.11	0.7±1.05 a	0.12±0.13 ab	0.05±0.05 ab	0.03±0.05 ab	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	CBQ67857.1|probable beta-glucosidase [Sporisorium reilianum SRZ2]	2.37E-31	60.9	66.7
CL163	Basidiomycota	0.06	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.17±0.16 a	0.35±0.52 a	0.0±0.0 a	0.0±0.0 a	ADV20959.1|Beta-glucosidase, putative [Cryptococcus gattii WM276]	1.68E-30	66.7	48.4
CL170	Ascomycota	0.04	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.02±0.04 a	0.28±0.65 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	6.34E-58	88.4	100
CL171	Ascomycota	0.04	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.02±0.04 a	0.0±0.0 a	0.05±0.12 a	0.27±0.65 a	AAP57755.1|Cel3b [Trichoderma reesei]	1.00E-55	92.6	98.9
CL174	Ascomycota	0.04	0.0±0.0 a	0.0±0.0 a	0.25±0.61 a	0.02±0.04 a	0.0±0.0 a	0.0±0.0 a	0.05±0.05 a	0.02±0.04 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	6.87E-59	89.5	100
CL176	Basidiomycota	0.04	0.0±0.0 b	0.0±0.0 b	0.27±0.35 a	0.03±0.05 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	CAE01320.1|beta glucosidase precursor [Uromyces viciae-fabae]	2.23E-41	63.2	63.6
CL178	Zygomycota	0.04	0.03±0.08 a	0.0±0.0 a	0.3±0.6 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	BAO00904.1|beta glucosidase 1 [Mucor javanicus]	2.93E-40	65.3	100
CL179	Ascomycota	0.04	0.02±0.04 a	0.0±0.0 a	0.28±0.47 a	0.02±0.04 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.30E-50	73.7	100
CL190	Ascomycota	0.04	0.0±0.0 a	0.33±0.82 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AAP57760.1|Cel3e [Trichoderma reesei]	2.03E-28	69.8	74.1
CL194	Ascomycota	0.05	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.27±0.51 a	0.17±0.09 a	0.0±0.0 a	0.03±0.05 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	9.44E-55	86.3	100
CL223	Ascomycota	0.04	0.25±0.48 a	0.0±0.0 a	0.06±0.16 a	0.03±0.05 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD50179.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	5.74E-46	80.2	100
CL242	Ascomycota	0.06	0.02±0.04 a	0.38±0.94 a	0.0±0.0 a	0.0±0.0 a	0.02±0.04 a	0.0±0.0 a	0.017±0.04 a	0.02±0.04 a	BAA19913.1|beta-D-glucosidase [Aspergillus kawachii]	1.88E-42	63	35.1
CL253	Ascomycota	0.04	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.27±0.55 a	0.03±0.05 a	0.0±0.0 a	0.0±0.0 a	CCD50179.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	1.16E-38	77.8	42.5
CL256	Ascomycota	0.11	0.22±0.53 a	0.63±1.55 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AAT95378.1|beta-glucosidase [Phaeosphaeria avenaria f. sp. tritici]	2.26E-53	80	100
CL257	Ascomycota	0.05	0.35±0.68 a	0.02±0.04 a	0.03±0.08 a	0.03±0.08 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AFU91382.1|GH3 beta-glucosidase [Talaromyces funiculosus]	3.94E-48	73.7	100
CL274	Ascomycota	0.09	0.68±1.67 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AER93027.1|beta-glucosidase, partial [Aspergillus aculeatus]	2.45E-38	65.3	100
CL283	Ascomycota	0.09	0.7±1.71 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	AEL79685.1|GH3 beta-glucosidase [Aspergillus saccharolyticus]	3.11E-41	69.5	100
CL325	Ascomycota	0.06	0.13±0.33 a	0.38±0.94 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD50179.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.16E-45	78	100
CL389	Ascomycota	0.05	0.37±0.9 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.02±0.04 a	0.02±0.04 a	CEF83026.1|unnamed protein product [Fusarium graminearum]	1.08E-44	75.8	48.4
CL398	Ascomycota	0.04	0.35±0.86 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CCD50891.1|glycoside hydrolase family 3 protein [Botrytis cinerea T4]	2.87E-60	91.6	100
CL499	Ascomycota	0.03	0.0±0.0 a	0.26±0.65 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	CEF83026.1|unnamed protein product [Fusarium graminearum]	1.11E-52	81.1	100

Chapter 5

"Microbial expression profiles in the rhizosphere of two maize lines differing in N use efficiency"

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Microbial expression profiles in the rhizosphere of two maize lines differing in N use efficiency

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Introduction

Rhizosphere is a peculiar soil microenvironment where soil properties, plant-roots and microorganisms characteristics and activities interact to each other in a coordinated manner. Rhizosphere hosts greater and more active microbial populations than the bulk soil, sustained by release of root exudates that stimulate microbial biomass and metabolic activities (Baudoin et al. 2003; Renella et al. 2007). Many beneficial microbes provide mineral nutrients and phyto-hormones to plants and also protects plants against phyto-pathogens in rhizosphere, some pathogenic microbes impair plant health (Marschner et al., 2004, Mendes et al., 2013). Therefore, interactions between plants and soil microorganism in the rhizosphere are of global importance to the rates of biogeochemical cycles, plant growth and crop productivity (Bloemberg and Lugtenberg, 2001; Philippot et al., 2009). It has been well established that plants select specific microbial groups in the rhizosphere in relation to the species, genotype, growth stage and soil properties (Berg and Smalla, 2009), but information on the functional activity expressed by the root-associate microbial communities is still poor. . It is therefore important to characterize the functional activity potential of rhizosphere microbial communities and link it to plant growth and physiological activity. It is estimated that currently only 1% of soil microorganisms is cultivable by traditional microbiological techniques. Development of culture independent techniques for identifying soil microorganisms by the analysis of nucleic acids, protein and metabolites provides new dimensions to our understanding of microbial diversity and their functions in soil. However, because the analysis of microbial genetic diversity mainly allows the inventory of all genes and the reconstruction of the potential metabolic pathways present in the soil microbial community, and seen the current limitations of soil proteomics caused by reduced protein extraction efficiency from soil (Giagnoni et al., 2011, 2012) whereas the analysis of the functions actually expressed by the soil microbial communities gene expression needs the analysis of gene expression (Urich et al., 2008; Carvalhais et al., 2012). Immediate regulatory

response to environment changes may be better reflected by the metatranscriptomic than the metaproteomic (Moran et al., 2009).

Maize (*Zea mays* L.) the 3rd most important crop after rice and wheat, being worldwide used for food, fodder and fibre production. Due to this reason, its root microbiome has been well characterized by high throughput pyrosequencing techniques (Dohrmann et al., 2013; Li et al., 2014a) and other methods (Chelius and Triplett, 2001; Aira et al., 2010; Li et al., 2014b). In a previous study on the microbial community composition, enzymatic activities and β-glucosidase encoding gene diversity in the rhizosphere of maize lines with different nitrogen use efficiency (NUE). It was reported that the maize line with higher NUE had a different microbial community, higher enzymatic activity than the low NUE maize line and induce a stronger selection of bacterial groups carrying β- glucosidase-encoding genes (Pathan et al., 2015a,b). It has been also demonstrated that the rhizosphere microbial communities and enzyme activities also change during the maize physiological responses upon the availability of different N forms (Giagnoni et al., 2015). However, information on the functions expressed by the rhizosphere microbial communities have not been well characterized.

In this study, comparative metatranscriptomic was used to study microbial expression profile in the rhizosphere of maize plants differing in their N use efficiencies. Main objectives were to relate the expression of functional genes in the rhizosphere during the growth of two maize lines differing their N use efficiency. We tested our hypotheses by studying the rhizosphere and bulk soil of maize plants with different NUE, grown in rhizoboxes allowing precise sampling of the rhizosphere and bulk soil. Best of our knowledge, this is first study of microbial expression analysis in rhizosphere of maize plants using mRNA metatranscriptomic approaches.

Material and Method

Soil properties, maize plants, and rhizobox setup

A sandy clay loam soil classified as a Eutric Cambisol (World Reference Base for Soil Resources 2006), under conventional maize crop regime, located at Cesa (Tuscany, Central Italy), was sampled from the Ap horizon (0–25 cm). The soil was sieved at field moisture (<2 mm), after removing visible plant material. Soil had a pH value (in H2O) of 7.1, contained 32.1 % sand, 42.2 % silt, 25.7 % clay, 10.8 g kg−1 total organic C (TOC), 1.12 g kg−1 total N, and 6.45 g kg−1 total P. After sieving, 600 g of soil was placed in the soil compartment of the rhizoboxes. The rhizoboxes consisted of two bulk soil compartments separated by the plant compartment, enclosed by 0.22-μm mesh nylon tissue. Full details on the used rhizoboxes and maize growth conditions have been already reported (Pathan et al.

2015a). The Lo5 and T250 maize lines, having high and low NUEs, respectively, were grown for 21 and 28 days, respectively, a suitable growth period to allow the full colonization of the plant compartment by plant roots and prevent nutrient starvation, as resulted from previous experiments (Pathan et al. 2015a). Plants were regularly watered with distilled sterile H2O, and no fertilizers were applied during the plant growth. All rhizoboxes were prepared in three replicates for each maize line. The used rhizoboxes allowed precise sampling of rhizosphere due to the presence of fixed sampling grooves at precise increment distances from the surface of the plant compartment. Rhizosphere (R) and bulk soil (B) samples of the Lo5 and T250 maize lines were named as Lo5R, Lo5B, T250R, and T250B, respectively. Rhizosphere and bulk samples were kept separate after sampling and stored at −80 °C before RNA extraction.

RNA extraction, rRNA removal and library preparation

RNA was extracted using the RNA PowerSoil Total RNA Isolation Kit (MOBio Laboratories). Five aliquots (5 x 1 g of soil) were extracted per sample and quintuplet RNA extracts were pooled together. Moreover, RNA was purified using RNeasy MinElute Cleanup Kit (QIAGEN). Extracted and cleaned RNA was treated with DNase I using the TURBO DNA-free Kit (ThermoFisher SCIENTIFIC).

This product was checked for quality (RIN number) and length distribution on an Agilent 2100 Bionalyser (Agilent Technologies). Approximately 100 ng of RNA was treated with an equimolar mixture of RiboZero rRNA Removal Kits Human-Mouse-Rat and Bacteria (Epicentre) to remove both prokaryotic and eukaryotic rRNA. rRNA removal was checked on an Agilent 2100 Bionalyser. Treated RNA served as the input for the ScriptSeq v2 RNA-Seq Library Preparation Kit (Epicentre), and the library size-distribution was re-checked on an Agilent 2100 Bionalyser (Agilent Technologies). Libraries were sequenced on an Illumina HiSeq2000 at the Argonne National Laboratory, USA, to generate 250-base paired-end reads.

Sequence data processing and analysis

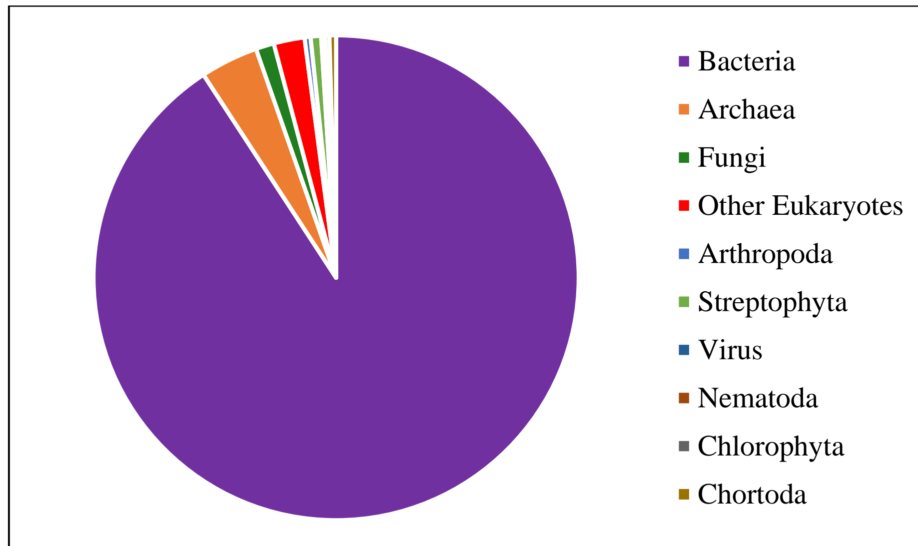
Generated metatranscriptomic sequences were processed as described by Žifčáková et al (2015). Briefly, reads were quality trimmed by removing adapters with Trimmomatic (v 477 0.27) using Illumina TruSeq2-PE adapters with a seed mismatch threshold, palindrome clip threshold, 478 and simple clip threshold set at 2, 30, and 10, respectively (Bolger et al., 2014). Furthermore, sequencing reads were filtered by base call quality using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html), specifically fastq_quality_filter, with the following parameters: -Q33 -q 30 -p 50. Resulting sequences were normalised using methods

previously described in (Howe et al., 2014, Pell et al., 2012) and Khmer (v 0.7.1) and command normalise-by-median.py with the following parameters: -k 20 -C 20 -N 4 -x 50e9. Next, errors were trimmed by removing low abundance fragments of high coverage reads with Khmer and command filter-abund.py -V. The paired-end assembly of the remaining reads was performed with the Velvet assembler (v 1.2.10, -exp_cov auto -cov_cutoff auto -scaffolding no (Zerbino and Birney 2008)) using odd k-mer lengths ranging from 33 to 63. Resulting assembled contigs were merged using CD-HIT v4.6 (Fu et al., 2012, Li and Godzik 2006) and minimus2 Amos v3.1.0 (Sommer et al., 2007). Broadly, protocols for this metatranscriptome assembly will be available at https://khmerprotocols.readthedocs.org/en/latest/mrnaseq/index.html. Sequence data of all contig sequences have been deposited in the MG RAST public database ((Meyer et al., 2008), http://metagenomics.anl.gov/, data set number 15341/Project TrainbiodiverseS). Contig annotation was performed in MG RAST with an E value threshold of 10^{-4}.

For the metatranscriptomic data, individual sequence reads from each sample were mapped onto contigs using bowtie 2.2.1 (Langmead et al., 2009) with the default settings of: end to end alignment –sensitive. The mapping was used to calculate transcript abundance, and data were expressed as: per base coverage = mapped read count x read length / contig length. Abundances were always reported as normalised values, i.e., shares of all transcripts in given sample, or, where indicated, shares of all transcripts of a selected microbial taxon. For the analysis of functional features, such as the KEGG categories, only those contigs belonging to archaea, bacteria and fungi and belonging to cellular processes, environmental information processing, genetic information processing and metabolism at the KEGG level 1 were considered for further analysis.

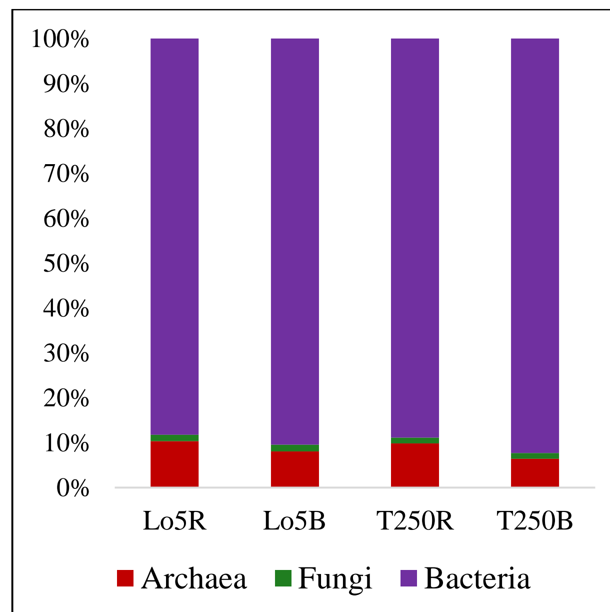
Preliminary Results

Metatranscpritomes were obtained, providing broader insights for comparisons of expressed functions in the rhizosphere of maize plants differing in their N use efficiencies. Sequences yielded 63614825 reads that were assembled into 558944 contigs over 200 bases and the longest contig had a length of 7645 bases. Features identified as microbial (i.e. those assigned to either Bacteria, Fungi or Archaea) represented a vast majority (95.86 %) of annotated contigs (figure 1). Of the other contigs, most had hits to Sterptophyta (0.7 %), Chortoda (0.43 %), Arthropoda (0.39 %) and Chlorophyta (0.29 %) (Figure 1). Whereas contigs identified as Virus represented only 0.05 % (Figure 1). Overall total of 1104 genus were identified in assembled contigs.



(Figure 1. Overall taxonomic assignment of assembled contigs)

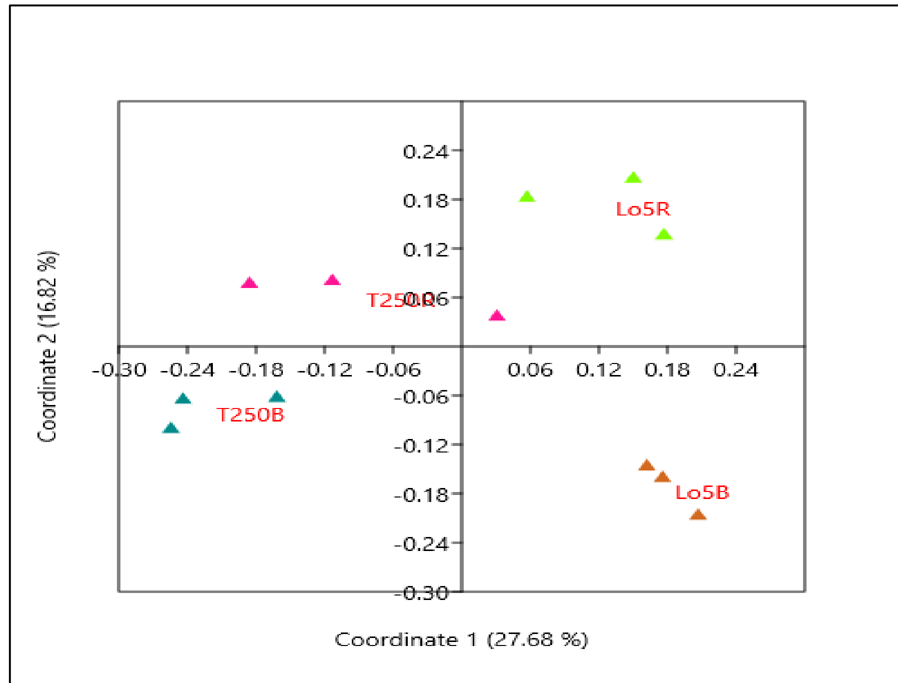
The contribution of microbial taxa to transcripts was same between rhizosphere and bulk soils of both maize lines. Approximately 88-92 % of transcripts were assigned to bacteria, 6-9 % to archaea and only 1 % of transcripts were assigned as Fungi (Figure 2).



(Figure 2. Contribution of Archaea, Bacteria and Fungi to transcripts in rhizosphere and bulk soil of maize lines Lo5 and T250)

The PCoA (Principal coordinates analysis) based on Bray-Curtis similarity matrix, was performed on contigs, which were annotated to Carbohydrate metabolism at KGGE level 2. Results of PCoA showed that rhizosphere soils were clearly clustered separately from the bulk soil of both maize lines. Contigs were annotated for β-glucosidase enzymes, which were involved in carbohydrate

metabolism, were shown into Table 1. Expressed β-glucosidase were mostly originated form Bacteroidetes, Firmicutes and Actinobacteria.



(Distribution of transcripts annotated for Carbohydrate metabolism in rhizosphere and bulk soils of Lo5 and T250 maize lines using PCoA based on Bray-Curtis similarity matrix)

Preliminary Discussion and Conclusion

Overall, above results indicated that bacteria were most dominant microbial group in rhizosphere of both maize lines, Lo5 and T250 but many archaea and fungal sequences were also detected in studied ecosystem. Rhizosphere does have effect on microbial functions, such as carbohydrate metabolism. In our pervious study, we found different phyla and species were harbouring β-glucosidase genes, such as Actinobacteria, Proteobacteria were most abundant phylum that carried potential β-glucosidase genes but representatives of Bacteroidetes, Chloroflexi, Deinococcus-Thermus, Firmicutes, and Cyanobacteria were also detected (Pathan et al., 2015b). However, metatranscriptomic data revealed that only few of them were active and expressing β-glucosidase genes, such as β-glucosidase genes from low abundant phyla, Bacteroidetes, Firmicutes were highly expressed compared to highly abundant phylum (Table 1). These indicate that even those genes showing low abundance may be functionally important as revealed by their high expression. Metatranscriptomic data analysis still going on to study overall microbial expression profile.

Contigs	Accession No	Microbial Group	Phylum	Species	E-values	Identified β-glucosidases
TR123938|c0_g1_i1	ACL69176.1	Bacteria	Firmicutes	*Aalothremothrix sp.*	2.40E-16	K01188; beta-glucosidase
TR123938|c0_g2_i1	ACL69176.1	Bacteria	Firmicutes	*Aalothremothrix sp.*	2.40E-16	K01188; beta-glucosidase
TR123938|c0_g3_i1	ACL69176.1	Bacteria	Firmicutes	*Aalothremothrix sp.*	2.40E-16	K01188; beta-glucosidase
TR138441|c0_g1_i1	ABG57307.1	Bacteria	Bacteroidetes	*Cytophyga hutchinsoni*	4.60E-11	K01188; beta-glucosidase
TR151692|c0_g2_i1	EAZ81398.1	Bacteria	Bacteroidetes	*Alyoziphagus machipongonesis*	7.50E-05	K01188; beta-glucosidase
TR151692|c0_g3_i1	EAZ81398.1	Bacteria	Bacteroidetes	*Alyoziphagus machipongonesis*	7.50E-05	K01188; beta-glucosidase
TR168351|c0_g1_i1	ADB30299.1	Bacteria	Actinobacteria	*Kribbella flavida*	7.50E-22	K01222 ; 6-phospho-beta-glucosidase
TR174331|c0_g1_i1	ADB30299.1	Bacteria	Actinobacteria	*Kribbella flavida*	4.30E-20	K01188; beta-glucosidase
TR18805|c0_g1_i1	EET14142.1	Bacteria	Bacteroidetes	*Bacteroidetes sp.*	8.40E-19	K01188; beta-glucosidase
TR19957|c0_g1_i1	ACY49489.1	Bacteria	Bacteroidetes	*Rhodothermus marinus*	2.90E-21	K01188; beta-glucosidase
TR375584|c0_g1_i1	EFL05146.1	Bacteria			6.60E-06	K01222 ; 6-phospho-beta-glucosidase
TR378816|c0_g1_i1	ADJ43109.1	Bacteria	Actinobacteria	*Amycolaptopsis mediterranie*	2.40E-33	K01188; beta-glucosidase
TR402736|c0_g1_i1	EFK62329.1	Bacteria	Bacteroidetes	*Parabacteroides sp.*	7.70E-26	K01188; beta-glucosidase
TR426405|c0_g1_i1	EFL05146.1	Bacteria	Actinobacteria	*Streptomyces sp.*	5.60E-20	K01222 ; 6-phospho-beta-glucosidase
TR426405|c0_g2_i1	EFL05146.1	Bacteria	Actinobacteria	*Streptomyces sp.*	5.60E-20	K01222 ; 6-phospho-beta-glucosidase
TR4470|c0_g1_i1	ADG68734.1	Bacteria	Planctomycetes	*Planctopirus limnophila*	2.30E-10	K01188; beta-glucosidase
TR53978|c0_g1_i1	EFI02569.1	Bacteria	Bacteroidetes	*Bacteroidetes sp.*	6.40E-19	K01188; beta-glucosidase
TR60935|c0_g1_i1	ABJ83649.1	Bacteria	Acidobacteria	*Candidatus salibacter*	1.10E-05	K01188; beta-glucosidase
TR94910|c0_g1_i1	EFK62329.1	Bacteria	Bacteroidetes	*Parabacteroides sp.*	8.00E-30	K01188; beta-glucosidase

(Table 1. Identified β-glucosidases based on KEGG class 1 and 2 from rhizosphere and bulk soils of maize lines Lo5 and T250)

References

Aira M, Gómea-Brandón M, Lazcano C, Bääth E, Domínguez J (2010) Plant genotype strongly modified the structure and growth of maize rhizosphere microbial communities. Soil Biol & Biochem 42: 2276-2281

Baudoin E, Benizri E, Guckert A (2003) Impact of artificial root exudates on the bacterial community structure in bulk soiland maize rhizosphere. Soil Biol Biochem 35:1183–1192

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:43–350

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics**:** btu170.

Carvalhais LC, Dennis PG, Tyson GW, Schenk PM (2012) Application of metatranscriptomics to soil environments. Journal of Microbiological methods 91:246-251

Chelius MK, Triplett EW (2001). The diversity of archaea and bacteria in association with the roots of Zea mays L. Microb Ecol 41: 252–263.

Dohrmann AB, Kuting M, Junemann S, Jaenicke S, Schluter A, Tebbe CC (2013) Importance of rare taxa for bacterial diversity in the rhizosphere of Bt- and conventional maize varieties. The ISME Journal 7: 37-49

Fu L, Niu B, Zhu Z, Wu S, Li W (2012) CD-HIT: accelerated for clustering the 582 next-generation sequencing data. Bioinformatics 28: 3150-3152

Giagnoni L, Magherini F, Landi L, Taghavi S, Modesti A, Bini L, Nannipieri P, Van der Lelie D, Renella G (2011) Extraction of microbial proteome from soil: potential and limitations assessed through a model study. European Journal of Soil Science 62:74-81

Giagnoni L, Magherini F, Landi L, Taghavi S, van der Lelie D, Puglia M, Bianchi L, Bini L, Nannipieri P, Renella G, Modesti A (2012) Soil solid phases effects on the proteomic analysis of *Cupriavidus metallidurans* CH34. Biol Fertil Soils 48:425-433

Giagnoni L, Pastorelli R, Mocali S, Arenella M, Nannipieri P, Renella G (2015) Availability of different nitrogen forms changes the microbial communities and enzyme activities in the rhizosphere of maize lines with different nitrogen use efficiency. Applied Soil Ecology, in press, doi:10.1016/j.apsoil.2015.09.004

Howe AC, Jansson JK, Malfatti SA, Tringe SG, Tiedje JM, Brown CT (2014) Tackling soil 601 diversity with the assembly of large, complex metagenomes. Proc Natl Acad Sci USA 111**:** 4904-4909

Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10: 10

Li X, Rui J, Xiong J, Li J, He Z, Zhou J, et al. (2014a) Functional Potential of Soil Microbial Communities in the Maize Rhizosphere. PLoS ONE 9(11): e112609. doi:10.1371/journal.pone.0112609

Li X, Rui J, Mao Y, Yannarell A, Mackie R (2014b) Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. Soil Biol Biochem 68: 392–401

Marschner P, Crowley D, Yang CH (2004) Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. Plant Soil 261: 199-208

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic and human pathogenic microorganisms. FEMS Microbiol Rev 37: 634-663

Moran, A.M., 2009. Metatranscriptomics: eavesdropping on complex microbial communities. Microbe 4, 329–335.

Pathan SI, CeccheriniMT, Pietramellara G, Puschenreiter M, Giagnoni L, Arenella M, Varanini Z, Nannpieri P, Renella G (2015a) Enzyme activity and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency. Plant Soil 387:413–424

Pathan SI, Ceccherini MT, Hansen MA, Giagnoni L, Ascher J, Arenella M, Sørensen SJ, Pietramellara G, Nannipieri P, Renella G (2015b) Maize lines with different Nitrogen Use Efficiency (NUE) also differ for molecular diversity of bacterial β-glucosidase gene and glucosidase activity in their rhizosphere. Biol Fertil Soils 51:995-1004

Pell J, Hintze A, Canino-Koning R, Howe A, Tiedje JM, Brown CT (2012) Scaling metagenome sequence assembly with probabilistic de Bruijn graphs. Proc Natl Acad Sci USA 109: 13272-13277

Renella G, Landi L, Valori F, Nannipieri P (2007) Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clayey and a sandy soil. Appl Soil Ecol 36:124–129

Sommer, D.D., Delcher, A.L., Salzberg, S.L., Pop, M. (2007) Minimus: a fast, lightweight genome assembler. BMC Bioinformatics 8: 64

Urich T, Lanzèn A, Qi J, Huson DH, Schleper C, et al. (2008) Simultaneous Assessment of Soil Microbial Community Structure and Function through Analysis of the Meta-Transcriptome. PLoS ONE 3(6): e2527. doi:10.1371/journal.pone.0002527

Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18: 821-829

Žifčáková L, Větrovsky T, Adina CH, Baldrian P (2015) Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Env. Microbiol. In Press

Chapter 6: Conclusion

The adopted Rhizobox experimental set up allowed to study the changes induced in chemical and parameters, enzyme activities, microbial biomass, microbial community composition and β-glucosidase encoding gene diversity in rhizosphere of Lo5 and T250 inbred maize lines, characterized by high and low NUE, respectively.

Lo5 maize line with high NUE induced faster inorganic N depletion in the rhizosphere and larger changes in in microbial biomass and different enzyme activities. Both lines induced differences in studied microbial community composition in their studied rhizosphere with large modification induced by high NUE lo5 maize line.

Our results also confirmed that plants can also select key functional genes such as those encoding β-glucosidase activity. Maize line with higher NUE induced a strong selection of bacterial β-glucosidase encoding genes and this selection could be related to higher C-hydrolysing enzyme activities in the rhizosphere. β-glucosidase encoding genes were mostly originated from most abundant phyla such as Actinobacteria and proteobacteria in rhizosphere of these maize lines but but representatives of Bacteroidetes, Chloroflexi, Deinococcus-Thermus, Firmicutes, and Cyanobacteria were also detected.

To our knowledge, these results are the first showing that the rhizospheres of plants differing in NUE are also characterized by differences in the diversity of key functional genes such as those encoding for the β-glucosidase activity. Soil chemical properties and biochemical activities strongly influenced the microbial community composition and β-glucosidase encoding genes diversity in rhizosphere of Lo5 and T250 maize lines.

Our overall results indicate the while plant genetic and physiological mechanisms are of fundamental importance to in their nutrient acquisition, they also show that maize lines with higher NUE also enhance microbial activities in the rhizosphere; therefore both factors likely concur the higher global plant NUE and should be maintained to pursuit best management practices in the agro-ecosystems. Future research is needed to characterize root exudates profile of studied maize lines to better explain the effect of root exudates on microbial community composition and on diversity of β-glucosidase encoding genes and determination of expressed protein to link diversity and expression.

Rhizobox experiment is still in progress, using mRNA metatranscriptomic approach to study microbial expression profile in the rhizosphere of maize plants differing in their N use efficiencies and to relate the expression of functional genes in the rhizosphere during the growth of two maize lines differing their N use efficiency. Best of our knowledge, this is first study of microbial expression analysis in rhizosphere of maize plants using mRNA metatranscriptomic approaches which provides further deeper knowledge on soil functional diversity.

Organic matter decomposition plays an important role in the carbon cycle in terrestrial environments including the globally widespread coniferous forests. Cellulose degradation is very important in this respect because cellulose represents one of the most abundant polysaccharides in the plant litter. β-glucosidases complete the final step of cellulose hydrolysis by converting cellobiose to simple glucose molecules and deliver glucose for the central metabolism.

In fungi, Ascomycota and Basidiomycota and in bacteria the phyla Firmicutes, Actinobacteria, Proteobacteria, Acidobacteria and Deinococcus-thermus represented the major reservoirs of β-glucosidase genes and indicated that a highly diverse microbial community may mediate cellobiose utilization in coniferous forests.

Seasonality does have influence on potential diversity of β-glucosidase genes but intense changes occurred in transcription profile. In fungi, DNA derived communities were overlapping among two seasons or two horizons, especially in litter horizon during both seasons but transcribes showed distinct association with either L or H horizons in summer and winter. In bacteria, distribution of the abundant OTUs between summer and winter for each horizon was strict confinement of many OTUs in RNA derived community, either in summer or in winter, especially in humic horizon.

The consensus sequences of bacterial and fungal β-glucosidase genes derived from this study differed and more diverse from previously published sequences, which were mainly based on cloning and sequencing of DGGE bands (Kellner et al., 2010; Caňizares et al., 2011; Li et al 2013). Hence, the depth of environmental amplicon sequencing may contribute to better evaluation of targeted functional gene diversity.

Overall, our results confirmed that seasonality is likely key driver of changes in β-glucosidase encoding gene diversity and their expression. Rich communities of both bacteria and fungi express β-glucosidase. Even those genes showing low abundance may be functionally important as revealed by their high expression. The functional diversity in the studied ecosystem seasons clearly exhibited a seasonal pattern but seasons did not have any kind of effect on β-glucosidase activity which

suggesting that analysis of gene expression at protein level is needed to better understand the link between diversity of β-glucosidase encoding gene and expressed β-glucosidase protein. Best of our knowledge, it is first time that we have studied phylogenetic distribution of single functional gene in bacteria and fungi, involved in cellulose decomposition using enough in depth sequencing in forest soils.

Finally, we can conclude that fungi and bacteria both are the main reservoirs of β-glucosidase genes and different biotic and abiotic factors such as seasonality, plant NUE, different soil conditions have strong influence on genetic dynamics of β-glucosidase genes and their expression. Despite the potential of genomic and transcriptomic to reveal dynamics of β-glucosidase encoding gene, results of these methods need to be verified by complementary approaches such as proteomics and direct isotopic labelling to link diversity of β-glucosidase encoding gene and their expression as protein.

References

Acosta-Martinez V, Klose S, and Zobeck TM (2003) Enzyme activities in semiarid soils under Conservation Reserve Program, native rangeland, and cropland. J. Plant Nutr. Soil Sci. 166:699–707.

Aira M, Gómea-Brandón M, Lazcano C, Bääth E, Domínguez J (2010) Plant genotype strongly modified the structure and growth of maize rhizosphere microbial communities. Soil Biol & Biochem 42: 2276-2281

Alef K and Nannipieri P (1995) Cellulase activity, methods in applied soil microbiology and biochemistry. Academic, London

Alef K and Nannipieri P (1995) Methods in Applied Soil Microbiology and Biochemistry. ISBN: 978-0-12-51380-6

Andrén O and Balandreau J (1999) Biodiversity and soil functioning: from black box to can of worms? Applied Soil Ecology 13: 105-108

Aon MA, and Colaneri AC (2001) Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. Appl. Soil Ecol. 18:255–270

Ascher J, Ceccherini MT, Pantani OL, Agnelli A, Borgogni F, Guerri G, Nannipieri P, Pietramellara G (2009) Sequential extraction and fingerprinting of soil metagenome Appl Soil Ecol 42: 176-181

Arnosti C (2011) Microbial Extracellular Enzymes and the Marine Carbon Cycle. Annual Review of Marine Science 3: 401-425

Badalucco L, Nannipieri P (2007) Nutrient transformation in the rhizosphere In Pinton R Varanini Z Nannipieri P (Eds) The rhizosphere Biochemistry and organic substances at the soil-plant interface, CRC Press Boca Raton, FL pp 111-133

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM, (2004) How plants communicate using the underground information superhighway. Tr Pl Sci 9: 26-32

Balconi C, Brosio D, Motto M (1997) Analysis of nitrogen partitioning in maize. Maize Gen Coop News 71: 10-11

Baldrian P & Valášková V (2008) Degradation of cellulose by basidiomycetous fungi. FEMS Microbiol Rev 32: 501–521.

Baldrian P, Kolařík M, Štursová M, Kopecky J, Valášková V, Větrovsky T, Žifčáková L, Anajdr J, Rídl J, Vlček C, Vaříšková J (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. The ISME Journal 6: 248-258

Baldrian P, Šnajdr J, Merhautova V, Dobiašova P, Cajthaml T, Valaškova V (2013) Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biol Biochem 56: 60-68

Bandick AK, Dick R (1999) Field management effects on soil enzyme activities. Soil Biology and Biochemistry 31:1471-1479

Bao, L, Huang Q, Chang L, Sun Q, Zhou J, Lu H (2012) Cloning and characterization of two beta-glucosidase/xylosidase enzymes from yak rumen metagenome. Appl. Biochem. Biotechnol. 166, 72–86

Basu U, Francis JL, Whittal RM, Stephens JL, Wang Y, Zaiane OR, Goebel R, Muench DG, Good AG, Taylor GJ (2006) Extracellular proteomes of *Arabidopsis thaliana* and *Brassica napus* roots: analysis and comparison by MudPIT and LC-MS/MS. Pl Soil 286: 357-376

Barrett T, Suresh CG, Tolley SP, Dodson EJ & Hughes MA (1995) The Crystal-Structure of a Cyanogenic Beta-Glucosidase from White Clover, a Family-1 Glycosyl Hydrolase Structure 3**:** 951-960

Barriuso J, Marín S, Mellado RP (2010) Effect of the herbicide glyphosate on glyphosate-tolerant maize rhizobacterial communities: a comparison with pre-emergency applied herbicide consisting of a combination of acetochlor and terbuthylazine. Environmental Microbiology 12:1021-1030

Baudoin E, Benizri E, Guckert A (2003) Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biol Biochem 35: 1183-1192

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68: 1-13

Berlemont R, Martini CA (2013) Phylogenetic distribution of potential cellulases in bacteria. Applied and Environmental Microbiology 79: 1545-1554

Blair G (1993) Nutrient efficiency - what do we really mean. In: Genetic Aspects of Plant Mineral Nutrition Randall PJ, Delhaize E, Richards RA, Munns R, (Eds.) Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 205-213

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:43–350

Bodelier PL (2011) Towards understanding, managing and protecting microbial ecosystems. Front. Microbio. 2:80. doi: 10.3389/fmicb.2011.00080

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics**:** btu170.

Bowen GD, Rovira A (1991) The rhizosphere. In: Walsel, Y., Eshel, A., Kafkafi, U. (Eds.), Plants Root, the Hidden Half. Marcel Dekker, New York, pp. 641-669

Bray JR and Curtis JT (1957) An ordination of upland forest communities of southern Wisconsin. Ecological Monographs 27:325-349

Bremer C, Braker G, Matthies D, Reuter A, Engels C, Conrad R (2007) Impact of plant functional group, plant species and sampling time on the composition of *nir*K-type denitifier communities in soil. Appl Environ Micrbio 73: 6876-6884

Bressan M, Roncato MA, Bellvert F, Comte G, Haichar FZ, Achouak W, Berge O (2009) Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. ISME J 3: 1243-1257

Briones AM, Okabe S, Umemiya Y, Ramsing N, Reichardt W, Okuyama H (2002) Influence of different cultivars on populations of ammonia-oxidizing bacteria in the root environment of rice. Appl Environ Microbiol 68: 3067-3075

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2007) Root exudates regulate soil fungal community composition and diversity. Appl Env Microbiol 74: 738-744

Browman MG, Tabatabai MA (1978) Phosphodiesterase activity of soils. Soil Sci Soc Am J 42: 284-290

Cakmacki, R, Kantar F, Algur OF (1999) Sugar beet and barley yields in relation to *Bacillus polymyxa* and *Bacillus megaterium* var. *phosphaticum* inoculation. Journal of Plant Nutrition and Soil Science 162:437-442

Cañizares R, Benitez E, Ogunseitan OA (2011) Molecular analyses of beta-glucosidase diversity and function in soil. European Journal of Soil Biology 47:1–8

Cañizares R, Moreno B, Benitez E (2012a). Biochemical characterization with detection and expression of bacterial β-glucosidase encoding genes of a Mediterranean soil under different long-term management practices. Biology and Fertility of Soils 48:651-663

Cañizares R, Moreno B, Benitez E (2012b) Bacterial β-glucosidase function and metabolic activity depend on soil management in semiarid rainfed agriculture. Ecology and Evolution 2:727-731

Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B., 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res. 37, 233–238

Carvalhais LC, Dennis PG, Tyson GW, Schenk PM (2012) Application of metatranscriptomics to soil environments. Journal of Microbiological methods 91:246-251

Cesco S, Mimmo T, Tonon G, Tomasi N, Pinton R, Terzano R, Neumann G, Weisskopf L, Renella G, Landi L, Nannipieri P (2012) Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. Biol Fertil Soils 48: 123-149

Ciardi C, Nannipieri P (1990) A comparison of methods for measuring ATP in soil. Soil Biology and Biochemistry 22:725-727

Chauhan PS, Chaudhry V, Mishra S, Nautiyal CS (2011) Uncultured bacterial diversity in tropical maize (*Zea mays* L.) rhizosphere. Journal of Basic Microbiology 51:15-32

Chhabra S, Brazil D, Morrissey J, Burke JI, O'Gara FN, Dowling D (2013) Characterization of mineral phosphate solubilization traits from a barley rhizosphere soil functional metagenome Microbiol Open 2: 717-724

Chelius MK, Triplett EW (2001) The diversity of Archaea and Bacteria in association with the roots of *Zea mays* L. Microbial Ecology 41:252-263

Chen M, Qin Y, Liu Z, Liu K, Wang F, Qu Y (2010) Isolation and characterization of a beta-glucosidase from Penicillium decumbens and improving hydrolysis of corncob residue by using it as cellulase supplementation. Enzyme Microb. Technol. 46:444–449

Chuenchor W, Pengthaisong S, Robinson RC *et al.* (2008) Structural insights into rice BGlu1 beta-glucosidase oligosaccharide hydrolysis and transglycosylation J Mol Biol **377:** 1200-1215

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117-143

Coleman DC, Odum EP, Crossley DA, (1992) Soil biology, soil ecology and global change. Biology and Fertility of Soils 14:104-111

Czjzek M, Cicek M, Zamboni V, Burmeister WP, Bevan DR, Henrissat B & Esen A (2001) Crystal structure of a monocotyledon (maize ZMGlu1) beta-glucosidase and a model of its complex with pnitrophenyl beta-D-thioglucoside. Biochem J. 354**:** 37-46

Davies GJ, Wilson KS & Henrissat B (1997) Nomenclature for sugar binding subsites in glycosyl hydrolases. Biochem J. 321**:** 557-559

de Giuseppe, PO et al (2014) Structural basis for glucose tolerance in GH1 beta-glucosidases. Acta Crystallogr D Biol Crystallogr. 70: 1631–1639

Deng SP, and Tabatabai MA (1996) Effect of tillage and residue management on enzyme activities in soils: 2. Glycosidases. Biol. Fertil. Soils 22:208–213

de la Horra AM, Conti ME and Palma RM (2003) β-Glucosidase and proteases activities as aff ected by long-term management practices in a Typic Argiudoll soil. Commun. Soil Sci. Plant Anal. 34:2395–2404

de Oliveira LG, Tormet Gonzalez GD, Samborsky M, Marcon J, Araujo WL, de Azevedo JL (2014) Genome sequence of Streptomyces wadayamensis strain A23, an endophytic actinobacterium from Citrus reticulata. Genome Announc. 2(4):e00625-14. doi:10.1128/genomeA.00625-14

Dohrmann AB, Kuting M, Junemann S, Jaenicke S, Schluter A, Tebbe CC (2013) Importance of rare taxa for bacterial diversity in the rhizosphere of Bt- and conventional maize varieties. The ISME Journal 7: 37-49

Duangmal K, Mingma R, Pathom-aree W, Thamchaipenet A, Inahashi Y, Matsumoto A, Takahashi Y (2011) Amycolatopsis samaneae sp. nov., isolated from roots of *Samanea saman* (Jacq.) Merr. International Journal of Systematic and Evolutionary Microbiology 61:951-955

Edwards IP, Zak DR (2010) Phylogenetic similarity and structure of Agaricomycotina communities across a forested landscape. Mol Ecol 19: 1469–1482

Eichlerová I, Homolka L, Žifčáková L, Lisá L, Dobiášová P, Baldrian P (2015) Enzymatic systems involved in decomposition refelects the ecology and taxonomy of saprotrophic fungi. Fungal Ecology 13: 10-22

Esen A (1993) b-glucosidases: overview. In: Esen A (ed) b-glucosidases and molecular biology. American Chemical Society, Washington, DC, pp 9–17

Eyzaguirre J, Hidalgo M & Leschot A (2005) Beta-glucosidases from Filamentous Fungi: Properties, Structure, and Applications. Handbook of Carbohydrate EngineeringAnonymous pp. 645- 685. Taylor and Francis Group, LLC

Faure D, Henrissat B, Ptacek D, Bekri MA, Vanderleyden J (2001) The celA gene, encoding a glycosyl hydrolase family 3 β-Glucosidase in *Azospirillum irakense*, is required for optimal growth on cellobiosides. Applied and Environmental Microbiology 67:2380-2383

Fitz WJ, Wenzel WW, Zhang H, Nurmi J, Štípek K, Fischerová Z, Schweiger P, Köllensperger G, Ma L.Q, Stingeder G (2003) Rhizosphere characteristics of the arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval efficiency. Environ Sci Technol, 37: 5008-5014

Fu L, Niu B, Zhu Z, Wu S, Li W (2012) CD-HIT: accelerated for clustering the 582 next-generation sequencing data. Bioinformatics 28: 3150-3152

García-Salamanca A, Molina-Henares AM, Dillewijn PV, Solano J, Pizarro-Tobías Roca A, Duque E, Ramos JL (2012) Bacterial diversity of rhizosphere of maize and surrounding carbonate-rich bulk soil. Microb Biotechnol 6: 34-44

Gianfreda L. & Bollag J.-M. 1996. Influence of natural and anthropogenic factors on enzyme activity in soil. In: Stotzky G. & Bollag J.-M. (eds.), Soil Biochemistry, Marcel Dekker, Inc., New York, pp. 123-193.

Gianfreda L & Rao MA (2011) Stabilising enzymes as synthetic complexes. In: Dick RP (Ed.). Methods of soil enzymology. Soil Science Society of America, Madison 9: 319–369.

Gunata Z, Vallier MJ (1999) Production of a highly glucose-tolerant extracellular beta-glucosidase by three Aspergillus strains. Biotechnol. Lett. 21: 219–223

Giagnoni L, Magherini F, Landi L, Taghavi S, Modesti A, Bini L, Nannipieri P, Van der Lelie D, Renella G (2011) Extraction of microbial proteome from soil: potential and limitations assessed through a model study. European Journal of Soil Science 62:74-81

Giagnoni L, Magherini F, Landi L, Taghavi S, van der Lelie D, Puglia M, Bianchi L, Bini L, Nannipieri P, Renella G, Modesti A (2012) Soil solid phases effects on the proteomic analysis of *Cupriavidus metallidurans* CH34. Biol Fertil Soils 48:425-433

Giagnoni L, Pastorelli R, Mocali S, Arenella M, Nannipieri P, Renella G (2015) Availability of different nitrogen forms changes the microbial communities and enzyme activities in the rhizosphere of maize lines with different nitrogen use efficiency. Applied Soil Ecology, in press, doi:10.1016/j.apsoil.2015.09.004

Gilbert GA, Knight JD, Vance CP, Allan DL (1999) Acid phosphatase activity in phosphorus-deficient white lupin roots. Pl Cell Environ 22: 801-810

Glanville H, Rousk J, Golyshin P, Jones DL (2012) Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. Soil Biol Biochem 48: 88-95

Gomes NCM, Heuer H, Schonfield J, Costa R, Hagler LM, Smalla K (2001) Bactreial diversity of rhizosphere of maize (*Zea mays*) grown in tropical soil studies by temperature gradient gel electrophoresis. Pl Soil 232: 167-180

Gomes NCM, Landi L, Smalla K, Nannipieri P, Brookes PC, Renella G (2010) Effect of Cd- and Zn-enriched sewage sludge on soil bacterial and fungal communities Ecotox Environ Saf 73: 1255-1263

Gonzalez-Franco AC, Deobald LA, Spivak A, Crawford DL (2003) Actinobacterial chitinase-like enzymes: profiles of rhizosphere versus non-rhizosphere isolates. Canadian Journal of Microbiology 49:683-698

Green VS, Stott DE, Cruz JC, Curi N (2007) Tillage impacts on soil biological activity and aggregation in a Brazilian Cerrado Oxisol. Soil Tillage Res. 92:114–121

Haichar FEZ, Achouak W, Christen R, Heulin T, Marol C, Marais MF, Mougel C, Ranjard L, Balesdent J & Berge O (2007) Identification of cellulolytic bacteria in soil by stable isotope probing. Environ Microbiol 9: 625–634

Hakulinen N, Paavilainen S, Korpela T & Rouvinen J (2000) The crystal structure of beta glucosidase from *Bacillus circulans* sp alkalophilus: Ability to form long polymeric assemblies J Struct Biol **129:** 69-79

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistic software package for education and data analysis. Palaeontol Electron 4:1–9

Harvey AJ, Hrmova M, De Gori R, Varghese JN & Fincher GB (2000) Comparative modeling of the three-dimensional structures of family 3 glycoside hydrolases Proteins-Structure Function and Genetics 41: 257-269

Hawes MC, Bengough G, Cassab G, Ponce G (2003) Root caps and rhizosphere. J. Pl Gr Reg 21: 352-367

Helal HM, Sauerbeck D (1989) Carbon turnover in the rhizosphere. Zeitschriftfur Pflanzenernahrung und Bodenkunde 152:211-216

Henrissat B, Callebaut I, Fabrega S, Lehn P, Mornon JP, Davies G (1995) Conserved catalytic machinery and the prediction of a common fold for several families of glycosyl hydrolases. Proc Natl Acad Sci USA 92:7090–7094

Henrissat B, Bairoch A (1996) Updating the sequence-based classification of glycosyl hydrolases. Biochem. J. 316:695–696

Henrissat B & Davies G (1997) Structural and sequence-based classification of glycoside hydrolases Curr Opin Struct Biol **7:** 637-644

Heuer H, Krsek M, Baker P, Smalla K, Wellington EMH (1997) Analysis of actinomycete communities by specific amplification of genes encoding 16s rRNA and gel electrophoretic saparation in denaturing gradients. Appl Environ Microbiol 63: 3233-3241

Hinsinger P, Plassard C, Tang CX, Jaillard B (2003) Origins of root mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Pl Soil 248: 43-59

Hongrittipun P, Youpensuk S, Rerkasem B (2014) Screening of nitrogen fixing endophytic bacteria in *Oryza sativa* L. Journal of Agricultural Science 6:66-74

Howe AC, Jansson JK, Malfatti SA, Tringe SG, Tiedje JM, Brown CT (2014) Tackling soil diversity with the assembly of large, complex metagenomes. Proc Natl Acad Sci USA 111**:** 4904-4909

Jenny H. (1941) Factors of soil formation: a system of quantitative pedology. McGraw-Hill Book Company, NY

Jenkins J, Lo Leggio L, Harris G, Pickersgill R (1995) Betaglucosidase, beta-galactosidase, family A cellulases, family F xylanases and two barley glycanases form a superfamily of enzymes with 8-fold beta/alpha architecture and with two conserved glutamates near the carboxy-terminal ends of betastrands four and seven. FEBS Lett 362:281–285

Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Computer Applications in the Biosciences 8: 275-282

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequences alignment based on fat Fourier transform. Nucleic Acid Research 30: 3059-3066

Kellner H, Vandenbol M (2010) Fungi unearthed: Transcripts encoding lignocellulolytic and

chitinolytic enzymes in forest soil. PLoS ONE 5(6): e10971

Knight TR, Dick RP (2004) Differentiating microbial and stabilized β-glucosidase activity relative to soil quality. Soil Biol Biochem 36: 2089–2096

Konstantinidis KT, Ramette A, Tiedje JM (2006) Toward a more robust assessment of intraspecies diversity, using fewer genetic markers. Applied and Environmental Microbiology 72:7286-7293

Krogh, K.B.R., Harris, P.V., Olsen, C.L., Johansen, K.S., Hojer-Pedersen, J., Borjesson, J., Olsson, L., 2010. Characterization and kinetic analysis of a thermostable GH3 beta-glucosidase from Penicillium brasilianum. Appl. Microbiol. Biotechnol. 86, 143–154

Landi L, Valori F, Ascher J, Renella G, Falchini L, Nannipieri P (2006) Root exudate effects on the bacterial communities CO2 evolution nitrogen transformations and ATP content of rhizosphere and bulk soils. Soil Biol Biochem 38: 509-516

Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10: 10

Li H, Xu X, Chen H, Zhang Y, Xu J, Wang J, Lu X (2013) Molecular analyses of the functional microbial community in composting by PCR-DGGE targeting the genes of the β-glucosidase. Bioresource Technology 134:51-58

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J et al. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol 173: 611–620

Li, W, Godzik A (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22: 1658-1659

Li X, Rui J, Xiong J, Li J, He Z, Zhou J, et al. (2014a) Functional Potential of Soil Microbial Communities in the Maize Rhizosphere. PLoS ONE 9(11): e112609. doi:10.1371/journal.pone.0112609

Li X, Rui J, Mao Y, Yannarell A, Mackie R (2014b) Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. Soil Biol Biochem 68: 392–401

Locci G, Santi S, Monte R, Pinton R, Varanini Z (2001) Involvement of plasma membrane H^+-ATPase in nitrate uptake by maize genotypes In: Horst W, Schenk MK, Bürkert A, Claassen N, Flessa H, Frommer WB, Goldbach HE, Olfs HW, Römheld V, Sattelmacher B, Schmidhalter U, Schubert S, von Wirén N, Wittenmayer L (Eds) Plant nutrition-Food security and sustainability of agro-ecosystems. Kluwer Academic Publishers Dordrecht pp 184-185

López-Mondéjar R, Voříšková J, Větrovský T, Baldrian P (2015). The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. Soil Biol Biochem 87: 43-50

Lugtenberg BJJ, Bloemberg GV (2004) Life in the rhizosphere. In: Ramos, J.L. (Ed) Pseudomonas. Kluwer Academic/Plenum Publishers, New York, NY (USA), pp. 403-430

Lombard V, Ramulu HG, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42: 490–495

Lynch JM, Benedetti A, Insam H, Nuti MP, Samalla K, Torsvik V, Nannipieri P (2004) Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plant and transgenic microorganisms. Biol Fertil Soils 40:363-385

Lynd LR, Weimer PJ, van Zyl WH & Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66: 506–577

Marschner P, Crowley D, Yang CH (2004) Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. Plant Soil 261: 199-208

McCaig AE, Glover LA, Prosser TI (1998) Molecular analysis of bacterial community structure under different land management regimes by using 16s ribosomal DNA sequence data and denaturing gradient gel electrophoresis banding patterns Appl Environ Microbiol 67: 4554-4559

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic and human pathogenic microorganisms. FEMS Microbiol Rev 37: 634-663

Metcalfe AC, Krsek M, Gooday GW, Prosser JI, Wellington EMH (2002) Molecular analysis of a bacterial chitinolytic community in an upland pasture. Applied and Environmental Microbiology 68:5042–5050

Miller HJ, Henken G, van Veen JA (1989) Variation and composition of bacterial populations in the rhizosphere of maize, wheat, and grass cultivars. Can J Microbiol 35: 656-660

Miller M, and RP Dick (1995) Th ermal stability and activities of soil enzymes as influenced by crop rotations. Soil Biol. Biochem. 27:1161–1166.

Moran, A.M., 2009. Metatranscriptomics: eavesdropping on complex microbial communities. Microbe 4, 329–335

Moreno B, Cañizares R, Nuñez R, Benitez E (2013) Genetic diversity of bacterial β-glucosidase encoding genes as a function of soil management. Biol Fertil Soils 49:735-745

Nam KH, Sung MW, Hwang KY (2010) Structural insights into the substrate recognition properties of β-glucosidase. Biochem and BioPhysic Research Communication 391: 1131-1135

Nannipieri P, Ceccanti B, Cervelli S, Sequi P (1974) Use of 01 M pyrophosphate to extract urease from a podzol. Soil Biol Biochem 6: 359-362

Nannipieri P, Ceccanti B, Grego S (1990) Ecological significance of the biological activity in soil. In Soil Biochemistry Vol. 6 (eds JM Bollag & G Stotzky), Marcel Dekker, New York, pp.293-355

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil funtions. Eur J Soil Sci 54:655-670

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In Bunemann EK, et al (Eds) Phosphorus in Action Soil Biology 26 Springer Verlag Berlin Heidelberg pp 215-241

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Fornasier F, Moscatelli MC, Marinari S (2012) Soil enzymology: Classical and Molecular approches. Biol Fertil Soil 48: 743-762

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B (2012) Soil enzymology: classical and molecular approaches. Biol Fertil Soils 48: 743-762

Neumann G, George TS, Plassard C (2009) Strategies and methods for studying the rhizosphere the plant science toolbox. Pl Soil 321: 431-456

Nijikken Y, Tsukada T, Igarashi K, Samejima M, Wakagi T, Shoun H & Fushinobu S (2007) Crystal structure of intracellular family 1 beta-glucosidase BGL1A from the basidiomycete *Phanerochaete chrysosporium* FEBS Lett 581: 1514-1520

Nübel U, Engelen B, Felske A, Anaidr J, Wieshber A, Amann RI, Ludwig W, Backhaus H (1996) Sequence heterogieties of genes encoding 16s rRNA in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis. J Bacteriol 178: 5636-5643

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. (2005) Fungal community analysis by large-scale sequencing of environmental samples. Appl Environ Microbiol 71: 5544–5550

Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Miller RH, Keeney DR (Eds) Methods of soil analysis 2nd edition Part 2 ASA-SSSA Madison WI USA pp 403-430

Pathan SI, CeccheriniMT, Pietramellara G, Puschenreiter M, Giagnoni L, Arenella M, Varanini Z, Nannpieri P, Renella G (2015a) Enzyme activity and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency. Plant Soil 387:413–424

Pathan SI, Ceccherini MT, Hansen MA, Giagnoni L, Ascher J, Arenella M, Sørensen SJ, Pietramellara G, Nannipieri P, Renella G (2015b) Maize lines with different Nitrogen Use Efficiency (NUE) also

differ for molecular diversity of bacterial β-glucosidase gene and glucosidase activity in their rhizosphere. Biol Fertil Soils 51:995-1004

Parameswaran P, Jalili R, Tao L, Shokralla S, Gharizadeh B, Ronaghi M et al. (2007) A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing. Nucleic Acids Res 35: e130

Pausch J, Tian J, Riederer M, Kuzyakov Y (2013) Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study. Pl Soil 364: 273-285

Pell J, Hintze A, Canino-Koning R, Howe A, Tiedje JM, Brown CT (2012) Scaling metagenome sequence assembly with probabilistic de Bruijn graphs. Proc Natl Acad Sci USA 109: 13272-13277

Perez-Pons JP, Cayetano A, Rebordosa X, Querol E (1994) A β-glucosidase gene (bgl3) from *Sterptomyces sp.* Strains QM-B814 molecular cloning, nucleotide sequence, purification and charachterization of the encoded enzyme, a new member of family 1 glycosyl hydrolases. Eur. J. Biochem. 233: 557-565

Pikuta E, Lysenko A, Chuvilskaya N, Mendrock U, Hippe H, Suzina N, Nikitin D, Osipov G, Laurinavichius K (2000) *Anoxybacillus pushchinensis* gen. nov., sp. nov., a novel anaerobic, alkaliphilic, moderately thermophilic bacterium from manure, and description of *Anoxybacillus flavithermus* comb. nov. International Journal of Systematic and Evolutionary Microbiology 50:2109-2117

Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol Lett 14: 187-194

Pozzo T, Pasten JL, Karlsson EN & Logan DT (2010) Structural and Functional Analyses of beta-Glucosidase 3B from *Thermotoga neapolitana*: A Thermostable Three-Domain Representative of Glycoside Hydrolase 3 J Mol Biol **397:** 724-739

Rao MA, Scelza R, Gianfreda L (2014) Soil enzymes. In: Gianfreda L, Rao MA (Ed.), Enzymes in Agricultural Sciences. Omics Group International, CA, pp. 1-18

Raun W, Gohnson GV (1999) Improving nitrogen use efficiency for cereal production. Agron J 91: 357-363

Raup DM, Crick RE (1979) Measurment of faunal similarity in paleontology. J Paleont 53: 1213-1227

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Renella G, Landi L, Valori F, Nannipieri P (2007) Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clayey and a sandy soil. Appl Soil Ecol 36: 124-129

Riou C, Salmon JM, Vallier MJ, Gunata Z, Barre P (1998) Purification, characterization, and substrate specificity of a novel highly glucose-tolerant beta-glucosidase from Aspergillus oryzae. Appl. Environ. Microbiol. 64: 3607–3614

Roldan A, JR Salinas-Garcia, MM Alguacil E Diaz, and Caravaca F (2005) Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. Geoderma 129:178–185

Ruggiero P, Dec J, Bollag JM (1996) Soil as a catalytic system. In: Stotzky G. & Bollag JM (eds.), Soil Biochemistry, Vol. 9, Marcel Dekker, New York, pp. 79-122

Sanz-Aparicio J, Hermoso JA, Martinez-Ripoll M, Lequerica JL & Polaina J (1998) Crystal structure of beta-glucosidase A from *Bacillus polymyxa*: Insights into the catalytic activity in family 1 glycosyl hydrolases J Mol Biol **275:** 491-502

Šnajdr, J., Valaškova, V., Merhautova, V., Herinkova, J., Cajthaml, T., Baldrian, P. (2008) Spatial variability of enzyme activities and microbial biomass in the upper layers of *Quercus petraea* forest soil. Soil Biol Biochem 40: 2068-2075

Sanguin H, Remenant B, Dechesne A, Thioulouse J, Vogel TM, Nesme X, Moënne-Loccoz Y, Grundmann G.L. (2006) Potential of a 16S rRNA-based taxonomic microarray for analyzing the rhizosphere effects of maize on *Agrobacterium* spp. and bacterial communities. Appl Environ Microbiol 72: 4302-4312

Sasaki C, Yokoyama A, Itoh Y, Hashimoto M, Watanabe T, Fukamizo T (2002) Comparative study of the reaction mechanism of family 18 chitinases from plants and microbes. Journal of Biochemistry 13:557-564

Schellenberger S, Kolb S & Drake HL (2010) Metabolic responses of novel cellulolytic and saccharolytic agricultural soil Bacteria to oxygen. Environ Microbiol 12: 845–861

Schmidt MI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DA, Nannipieri P, Rasse DP, Weiner S, Trumbore SE (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49-56

Shannon CE, Weaver W (1948) A mathematical theory of communication The Bell System Technical Journal 27379–423

Shewale JG (1982) Beta-Glucosidase - its Role in Cellulase Synthesis and Hydrolysis of Cellulose Int J Biochem 14: 435-443

Simpson EH (1949) Measurement of diversity. Nature 163: 688

Singhania, R.R., et al. Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. Bioresour. Technol. (2012) http://dx.doi.org/10.1016/j.biortech.2012.09.012

Sharma A, Divya MS, Goyal DS, Mishra M, Shukla P (2014) Effect of soil on microbial diversity of flora and fauna in ecosystem. Recent Research in Science and Technology 6:188-190

Smalla K, Wieland G, Buchner A, Zock A, Parry J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: Plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67: 4742-4751

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85: 591-602

Sommer DD, Delcher AL, Salzberg SL, Pop M (2007) Minimus: a fast, lightweight genome assembler. BMC Bioinformatics 8: 64

Sørensen T (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. Kongelige Danske Videnskabernes Selskab 5:1-34

Stott DE, Andrew SS, Liebig MA, Wienhold BJ, Karlen DL (2010) Evaluation of β-glucosidase activity as a soil quality indicator for the soil management assessment framework. Soil Sci. Soc. Am. J. 74: 107-119

Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P (2012) Cellulose utilization in forest litter and soil: Identification of bacterial cellulose decomposers. FEMS Microbiol Ecol 80: 735-746

Sue M, Yamazaki K, Yajima S, Nomura T, Matsukawa T, Iwamura H & Miyamoto T (2006) Molecular and structural characterization of hexameric beta-D-glucosidases in wheat and rye PlantPhysiol 141**:** 1237-1247

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1: 301-307

Tabatabai MA (1982) Soil enzymes. In: Page AL, Miller RH, Keeney DR (Eds) Methods of Soil Analysis Part 2 Chemical and Microbiological Properties 2nd Ed American Society of Agronomy/Soil Science Society of America Madison WI pp 903-947

Tate RL (1995) Soil microbiology. John Wiley, New York

Tamura K, Stecher G, Peterson D, Filipski A, Kumar s (2013) MEGA 6: Molecular Evolutionary Genetic Analysis Version 6.0. Molecular Biology and Evolution 30: 275-2729

Ter Braak CJF, Smilaauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power Ithaca, Availabe at http://www.canoco.com

Thanh DTN, Diep CN (2014) Isolation, characterization and identification of endophytic bacteria in maize (*Zea mays* L.) cultivated on Acrisols of the Southeast of Vietnam. American Journal of Life Sciences 2:224-233

Tomscha JL, Trull MC, Deikman J, Lynch JP, Guiltinan MJ (2004) Phosphatase under-producer mutants have altered phosphorus relations. Pl Physiol 135: 334-345

Tomasi N, Weisskopf L, Renella G, Landi L, Pinton R, Varanini Z, Nannipieri P, Torrent J, Martinoia E, Cesco S (2008) Flavonoids of white lupin roots participate in phosphorus mobilization from soil. Soil Biol Biochem 40: 1971-1974

Tsukada T, Igarashi K, Yoshid M, Samejima M (2006) Molecular cloning and characterization of two intracellular beta-glucosidases belonging to glycoside hydrolase family 1 from the basidiomycete Phanerochaete chrysosporium. Appl. Microbiol. Biotechnol. 73: 807–814

Turbé A, De Toni A, Benito P, Lavelle P, Lavelle P, Ruiz N, Van der Putten WH, Labouze E, Mudgal S (2010) Soil biodiversity: functions, threats and tools for policy makers. Report for European Commission (DG Environment), Bio Intelligence Service, Paris

Turner BL, Hopkins DW, Haygarth, PM, Ostle N (2002) β-Glucosidase activity in pasture soils. Appl. Soil Ecol. 20:157–162

Turner, T.R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D. et al. (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME J 7**:** 2248-2258

Ulrich A, Klimke G, Wirth S (2008) Diversity and activity of cellulose-decomposing bacteria, isolated from a sandy and a loamy soil after long-term manure application. Microb Ecol 55: 512–522

Urich T, Lanzèn A, Qi J, Huson DH, Schleper C, et al. (2008) Simultaneous Assessment of Soil Microbial Community Structure and Function through Analysis of the Meta-Transcriptome. PLoS ONE 3(6): e2527. doi:10.1371/journal.pone.0002527

Uren NC (2007) Types amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R Varanini Z Nannipieri P (Eds) The Rhizosphere Biochemistry and Organic Substances at the Soil-Plant Interface Taylor and Francis Group Boca Raton FL pp 1-21

Uroz S, Buée M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environmental Microbiology Reports 2:281-288

Vainio EJ, Hantula J (2001) Genetic differntation between European and North American population of *Phlebiopsis gigantean*. Micologia 92: 436-446

Vaříšková J, Brabcová V, Cajthaml T, Baldrian P (2014) Seasonal dynamics of fungal communities in a temperate Oak forest soil. New Phytol 201: 269-278

Varghese JN, Hrmova M & Fincher GB (1999) Three-dimensional structure of a barley beta-D-glucan exohydrolase, a family 3 glycosyl hydrolase Structure **7:** 179-190.

Varghese JN, Hrmova M, Fincher GB (1999) Three-dimensional structure of a barley b-D-glucan exohydrolase; a family 3 glycosyl hydrolase. Structure 7:179–190

Větrovsky, T., Baldrian, P. (2013) Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED. Biol Fertil Soils 49:1027-1037

Wallenstein MD, Weintraub MN (2008) Emerging tools for measuring and modeling the *in situ* activity of soil extracellular enzymes. Soil Biol Biochem 40: 2098-2106.

Wang XQ, He XY, Yang SJ, An XM, Chang WR & Liang DC (2003) Structural basis for thermostability of beta-glycosidase from the thermophilic eubacterium *Thermus nonproteolyticus* HG102 J Bacteriol **185:** 4248-4255

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci 63: 251-263

Wenzel WW, Wieshammer G, Fitz WJ, Puschenreiter M (2001) Novel rhizobox design to assess rhizosphere characteristics at high spatial resolution. Pl Soil 237: 37-45

Williams C, and Jochem FJ (2006) Ectoenzyme kinetics in Florida Bay: Implications for bacterial carbon source and nutrient status. Hydrobiologia 569: 113–127

Wirth S & Ulrich A (2002) Cellulose-degrading potentials and phylogenetic classification of carboxymethyl-cellulosdecomposing bacteria isolated from soil. Syst Appl Microbio 25: 584–591

World Reference Base for Soil Resources, 2006. A Framework for International Classification, Correlation and Communication. World Soil Resources Reports No. 103. FAO, Rome, 116 pp

WRB World Reference Base for Soil Resources 1998 World Soil Resources Reports 84 FAO Rome

Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency Annu Rev Pl Biol 63: 153-182

Xu XW, Huo YY, Wang CS, Oren A, Cui HL, Vedler E, Wu M (2011) *Pelagibacterium halotolerans* gen. nov., sp nov and *Pelagibacterium luteolum* sp nov., novel members of the family Hyphomicrobiaceae. International Journal of Systematic and Evolutionary Microbiology 61:1817-1822

Zamboni A, Astolfi S, Zuchi S, Pii Y, Guardini K, Tononi P, Varanini Z (2014) Nitrate induction triggers different transcriptional changes in a high and a low nitrogen use efficiency maize inbred line. Journal of Integrative Plant Biology 56:1080-1094

Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18: 821-829

Žifčáková L, Větrovsky T, Adina CH, Baldrian P. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Env. Microbiol. In Press

Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaver AK (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Applied and Environmental Microbiology 68:2198-2208

Zornoza R, Landi L, Nannipieri P, Renella G (2009) A protocol for the assay of arylesterase activity in soil. Soil Biol Biochem 41: 659-662

Appendix 1: List of Abbreviations

ANOVA	:	Analysis of Variance
ANOSIM	:	Analysis of Similarity
ATP	:	Adenosine triphosphate
C	:	Carbon
CAZY	:	Carbohydrate Active enzyme
CBH	:	Cellobiohydrolases
CCA	:	Canonical Correspondence Analysis
cDNA	:	Complementary DNA
CE	:	Carbohydrate Esterase
CMCase	:	Carboxy Methyl Cellulase
DGGE	:	Denaturing Gradient Gel Electrophoresis
DNA	:	Deoxy Ribonucleic Acid
EDTA	:	Ethylene Diamine Tetra Acetic Acid
GH	:	Glycosyl Hydrolase
GH1	:	Glycosyl Hydrolase Family1
GH3	:	Glycosyl Hydrolase Family1
GT	:	Glycosyltransferases
HCA	:	Hydrophobic Cluster Analysis
JGI	:	Joint Genome Institute
LMWOCs	:	Low Molecular Weight Organic Compounds
ML	:	Maximum-Likelihood
mRNA	:	messenger RNA
MUB	:	Modified Universal Buffer
N	:	Nitrogen
NAGase	:	N-acetyl-glucosaminidase
NH_4^+-N	:	Ammonium

NO_3^- -N	:	Nitrate
NSI	:	Nucleotide Sequence Identity
NUE	:	Nitrogen Use Efficiency
OTU	:	Operational Taxonomic Unit
P	:	Phosphorus
PCR	:	Polymerase chain reaction
PCoA	:	Principal Coordinates Analysis
PL	:	Polysaccharide Lyases
p-NP	:	p-nitrophenol
qPCR	:	quantitative PCR
RNA	:	Ribonucleic Acid
RDA	:	Redundancy analysis
SIP	:	Stable Isotope Probes
SOC	:	Soil Organic Carbon
SOM	:	Soil Organic Matter
TOC	:	Total Organic Carbon
TAE	:	Tris-acetate-EDTA
UPGMA	:	Unweighted Pair Group Method with Arithmetical averages