Review

Perspective on the status and behaviour of SARS-CoV-2 in soil

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ABSTRACT

Soil contamination by SARS-CoV-2 is highly probable because soil can collect several transporters of the virus, such as fallout aerosols, wastewaters, relatively purified sludges, and organic residues. However, the fate and status of SARS-CoV-2 in soil and the possible risks for human health through contaminated food are unknown. Therefore, this perspective paper discusses the challenges of determining the SARS-CoV-2 in soil and the mechanisms concerning its adsorption, movement, and infectivity in soil, considering what has already been reported by perspective papers published up to May 2021. These issues are discussed, drawing attention to the soil virus bibliography and considering the chemical structure of the virus. The mechanistic understanding of the status and behavior of SARS-CoV-2 in soil requires setting up an accurate determination method. In addition, future researches should provide insights into i) plant uptake and movement inside the plant, ii) virus adsorption and desorption in soil with the relative infectivity, and iii) its effects on soil functions. Models should simulate spatial localization of virus in the soil matrix.

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1. Introduction

The soils host a remarkable variety of biodiversity relevant to fertility induction (Kuzyakov and Mason-Jones, 2018), including human pathogens. These pathogens are geobionts and geophites and are thus defined as euedaphic and soil-transmitted (ST) pathogens (Jeffery and Van Der Putten, 2011). For most ST pathogens,

soil acts as a refuge, allowing them to survive for a long time before infecting humans by utilizing soil particles as vectors. Humans affecting enteroviruses are capable to actively survive even till 100 days in soil (Duboise et al., 1976). Cases of infection with ST pathogens viruses are cited in the literature (Klein et al., 2020), including also those from zoonotic ones such as "hantavirus pulmonary syndrome" (HPS) or "hemorrhagic fever with renal syndrome" (HFRS) (Jonsson et al., 2010). These data, in addition to those related to the SARS Cov-2 survival in water (La Rosa et al., 2020), sludges (Peccia et al., 2020; Sims and Kasprzyk-Hordern, 2020; Sun et al., 2020; Tran et al., 2021), and human stool (Sun et al., 2020) make reasonable to hypothesize its presence and persistence in soil via contaminated water and atmospheric particles fallout (Anand et al., 2021). These aspects justify the interest in deepening our knowledge on SARS Cov-2 fate in the soil also in the light of the higher infectivity that seems increased in the latest detected variants.

We can speculate that the risks for human health by SARS-CoV-2 in soil are negligible because the virus charge of any breathed and contaminated soil particles are likely low. In addition, the risk of ingesting contaminated food (particularly fresh horticulture products irrigated with contaminated waters) may not be harmful because the stomach's acidity can destroy the virus. However, the fate and behaviour of SARS-CoV-2 in the soil are poorly known. There is no research on the presence and survival of SARS-CoV-2 in the soil, whereas some perspective papers have been published in the last two years. Analysis of soil (Conde-Cid et al., 2021a) and liquid samples from soil (Conde-Cid et al., 2021b) were mainly discussed for what concerns sampling, handling, and storing. At the same time, the qPCR approach is proposed for SARS-CoV-2 quantification. Interactions with soil surface-reactive particles were discussed by (Anand et al., 2021), underlining the critical role of the lipid membrane of the capsid in the virus adsorption in soil. The elution of the virus from soil was also discussed, the following virus concentration in the liquid samples, and the final quantification. We have stopped our bibliographic search in May 2021. This perspective contribution aims to improve the discussion of SARS-CoV-2 in soil by considering what is known about the persistence and infectivity of viruses in soil, which has been ignored in the past previous perspective studies. According to the format of perspective papers the number of citations is limited, and for this reasons, reviews are cited more than article papers.

2. Effects on soil functions, interactions with soil particles, and maintenance of infectivity

2.1. Brief state-of-the-art about soil viruses

Virus abundance can reach the number of 10^{10} g^{-1} soil, being 1–2 orders of magnitude higher than the bacterial number, and it depends on soil properties and virus characteristics (Williamson et al., 2017). Despite the fact that most soil viruses are bacteriophages, their interactions with the soil microbiome are poorly studied (Kuzyakov and Mason-Jones, 2018). Viruses can have both positive and negative effects on the soil microbiome; for example, bacteriophages; can infect pathogenic bacteria as well as plant beneficial bacteria, such as rhizobia (Williamson et al., 2017). The rot incidence of potato tubers by *Dicheya solani* was reduced by LIME stone 1 and LIME stone 2, two phages lysing the pathogen (Pratama and van Elsas, 2019). Virulent bacteriophages can replicate and be released once they have infected the bacterial cells. In contrast, the genome of lysogenic bacteriophages is integrated into the host genome, remaining silent, thus without producing virions (Kuzyakov and Mason-Jones, 2018). Soil microorganisms can inactivate viruses (Nasser et al., 2002; Reyes et al., 2004) or

use viruses as energy and nutrient sources (Stotzky, 1986) also through the release of extracellular enzymes degrading virus molecules (Nasser et al., 2002; Reyes et al., 2004). Pratama and van Elsas (Pratama and van Elsas, 2019) has hypothesized that bacteriophages lower the abundance of bacteria in soil hotspot. Still, at low bacterial abundance values, the phage-host cell encounters markedly decreases, which may allow bacterial growth with the consequent increase in bacterial abundance, giving a temporal fluctuation in bacterial abundance.

The virus distribution in the soil matrix plays an important role in the interactions of viruses with soil microorganisms. Viruses being 10–100 times smaller than bacteria can occupy all soil pores larger than nano-pores, but likely bacteriophages are abundant where bacterial colonies are localized in the soil matrix (Kuzyakov and Mason-Jones, 2018). Viruses occupying micropores of micro-aggregates may move slower than those occupying mesopore, if they are not adsorbed to soil particles because water moves faster in mesopores than micro-pores. Noteworthy, viruses tend to occupy the thin soil layer under drought conditions. Drying increases the solution ionic strength, thus increasing the hydrophobicity of the surface of some viruses, which may affect virus adsorption to soil particles (Lance and Gerba, 1984).

The presence of metal oxides can inactivate viruses (Schijven and Hassanizadeh, 2002). Both humic acids and fulvic acids adsorb viruses removing them from infected cell surfaces with physico-chemical properties of humic acids and virus playing a role in the adsorption strength (Zhernov et al., 2021, 2017). The attachment of viruses to soil organic particles is highly probable because soil organic matter shows both hydrophilic and hydrophobic binding sites for viruses (Schijven and Hassanizadeh, 2002). On the contrary, the Dissolved Organic Matter (DOM) decreased the virus attachment by competing for the same binding sites and also disrupting hydrophobic bonds between viruses and soil particles (Schijven and Hassanizadeh, 2002). These contrasting effects of organic matter may explain the challenge in predicting the virus diffusion in organic soils (Schijven and Hassanizadeh, 2002).

Experiments with clay minerals have given insights into mechanisms responsible for the adsorption of viruses to surface-reactive soil particles and their inactivation once released, two bacteriophages of *E. coli*, T7 and T1 (both containing-stranded DNA and with an M of 20 × 106 and 25 × 106, respectively), one reovirus type 3 (containing double-stranded RNA and with an M of 70 × 106), and one herpesvirus hominis type 1 (HSV 1) (containing double-stranded DNA and with an M of 20 × 106) were adsorbed on montmorillonite or kaolinite (Stotzky, 1986). The adsorption depended on both clay minerals and virus types. For example, T7 had a greater affinity than T1 for both clay minerals, but each bacteriophage had a greater affinity for montmorillonite than kaolinite. However, they behaved differently because T7 adsorbed more negatively than positively charged sites of both clays, whereas T1 adsorbed mainly to positively charged sites on kaolinite and both negatively and positively charged sites of montmorillonite. Therefore, virus properties and soil pH (Gerba, 1984; Schijven and Hassanizadeh, 2002; Stotzky, 1986) can affect the adsorption process because some charges are pH-dependent on the surface-reactive soil particles. Changes in the charge sign can also occur on virus proteins. The protonation of the capsomere proteins was likely involved in the adsorption of the mainly negatively charged reovirus type 3 to kaolinite and montomorillonite, both clays being mostly negatively charged at neutral pH value of the experiment (Stotzky, 1986). Alkaline pH values can favor the leaching of viruses in soil due to repulsion between net negative surface charges of viruses and soil particles (Schijven and Hassanizadeh, 2002). However, this may also depend on the absence or scarce contribution of H bonding and van del Waals forces, which can also be involved in the adsorption of viruses to soil particles (Stotzky,

1986). The valency of the cation saturating the negative charges on surface-reactive soil particles is also vital because virus adsorption increased with the valency of the cation of the exchange complex of montmorillonite (Stotzky, 1986). The adsorption also depends on the presence of organic compounds; for example, amino acids or penicillin reduced the adsorption of T1 but not that of reovirus type 3 to montmorillonite because the former was adsorbed to positively charged sites and the latter to negatively charged sites (Stotzky, 1986). For this reason, there was no competitive adsorption between the reovirus and the bacteriophage to montmorillonite.

The adsorption or binding of viruses to soil particles can affect infectivity once the virus is desorbed from these particles. However, both bacteriopahges (T1 and T7) and reoviruses type 3 retained their infectivity once they were desorbed from clay minerals (Stotzky, 1986). In addition, the adsorption of the reovirus to clay minerals reduced the inactivation by estuarine water (Stotzky, 1986). Noteworthy, the capsid proteins of viruses exposed to telluric air can change conformation with the virus inactivation (Thompson et al., 1998).

3. What about SARS-CoV-2?

SARS-CoV-2 is an enveloped RNA virus prone to mutation, and it may be short-lived (a few days) in soil because of the proteic envelo, which may interact with surface-reactive soil particles (Klein et al., 2020). However, nucleic acids of the virus without an envelope can also interact with soil particles, thus being adsorbed (Pietramellara et al., 2009). Both proteases and nucleases are active in sol (Nannipieri et al., 2012), and thus either exposed proteins or exposed nucleic acids can be degraded. In addition, there was contrasting evidence about the resistance of SARS-CoV-2 to environmental stresses because the capsid is resistant (Katz et al., 2018). In contrast, Kiss et al (Kiss et al., 2021) reported that the capsid is fragile. The high relative soil moisture is unfavorable to the infectivity of HCoV 229E, HCoV OC43, viruses with similar properties of SARS-CoV-2, at a temperature higher than 6 °C (Geller et al., 2012).

Anand et al (Anand et al., 2021) underlined the role of the lipid membrane in the SARS-CoV-2 adsorption in soil. If this hypothesis is true, then hydrophilic surfaces of soil particles should be involved in the virus adsorption. However, the capsid of both SARS-CoV and SARS-CoV-2 shows positive charges (Gussow et al., 2020), thus making these viruses sensible to adsorption in soil due to the prevalence of negative charges over positive charges on the surface-reactive soil particles, especially at neutrality or under alkaline conditions. The viral capsid is composed of four proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) protein (Malik, 2020). Protein M defines the shape of the viral envelope, and protein S is responsible for the attachment. Then the entry of the virus in the host cell and the proteins E and N are responsible for the viral assembly, budding inside the host cell, and binding to the RNA genome (Malik, 2020). The N protein has a molecular weight of 46 kDa and an N-terminal region mainly consisting of positively charged amino acids, those involved in the RNA binding. Likely, this protein should not be involved in the virus adsorption by surface-reactive soil particles because it is located inside the capsid (Surjit and Lal, 2008). Both M and E proteins are embedded in the viral capsid (Malik, 2020), which may avoid their interaction with soil components. The S glycoprotein is composed of two associated heptad repeat regions, termed HR-N and HR-C, that form a very stable alpha-helical coiled structure characterized by a hydrophobic transmembrane anchor (HR2) and an hydrophic external structure (HR1[P1]; the S protein can show either an active or inactive conformation (Tripet et al.,

2004). The HR1 external exposure makes this protein the primary site of interaction between the virus capsid and the surface-reactive soil particles. When the protein is damaged, the hydrophobic moieties prevail over the hydrophic moieties (Schoeman et al., 2020), and the damaged protein can interact with the hydrophobic surfaces of soil particles. Luisetto et al (Luisetto et al., 2021) hypothesized that the SARS CoV- 2 is easily adsorbed by surface-reactive soil particles because of its large size and presence of positive and negative patches as well as hydrophobic and hydrophilic moieties (Fig. 1).

It is unknown if the adsorption of the SARS-CoV-2 on surface-reactive soil particles and the successive release affect the virus infectivity, also considering the contradictory opinion on capsid fragility (Katz et al., 2018; Kiss et al., 2021). We have summarized the driving forces that mainly affect the SARS-Cov-2 behavior in the soil concerning this aspect (Fig. 2). The presence of organic substances in the environment can also play a role in the preservation of the virus integrity because, for example, the virus lost its infectivity after interaction of the HR regions of the S protein with reactive humic and fulvic acids, which changed the protein conformation (Kotwal, 2008). The SARS CoV-2 is sensitive to pH values because the nucleocapsid protein begins to unfold at a pH near 5.0, and is denatured at pH 2.7 (Wang et al., 2004). However, the pH value of the microenvironment where the virus is located is important because the microenvironment pH may differ from the soil pH. Neutral to alkaline soils might favor epidemic outbreaks concerning acid soils because viruses are desorbed from soil particles due to repulsion between negative charges of virus and soil particle surfaces unless divalent and trivalent cations saturating the negative charges on the surface of soil particles can act as bridges and keep the virus on soil particles (Stotzky, 1986). Of course, this occurs when hydrophobic interactions are not important for the virus adsorption to soil particles.

4. The detection of the SARS-COV-2 in soil

As with any study in soil science, the analysis of main soil properties should precede the study of the SARS-COV-2 to relate the virus detection and infectivity to the soil type (Conde-Cid et al., 2021b). Other important issues are; i) the use and location of soil; ii) the sampling strategy and storage of soil samples; iii) the analysis of the viral sequences. Indeed, virus sources (use of wastewater for irrigation, organic fertilizers or sludge, etc.) may provide information on the contamination period of arable soils. The same is true for any human activity being a virus source to the target soil (Agnoletti et al., 2020).

The soil sampling strategy should consider the soil layer, surface and/or deeper layers to be collected, and rhizosphere and bulk soil to study the effects of plant roots on the virus (Pratama and van Elsas, 2019). As already mentioned, plants can mobilize viruses in soil by the rhizosphere effects (REs) through the release of root exudates (Schoeman et al., 2020; Swanson et al., 2009). The distribution of the virus in the soil matrix may also involve separating soil in aggregates with the detection of the virus in micro-, meso- and macro-aggregates.

As reviewed by Williamson et al (Williamson et al., 2017) and Pratama and van Elsas (Pratama and van Elsas, 2019), soil viriome analysis by the currently available DNA sequencing methods gives deeper taxonomic data and are more accurate than cultivation and morphological (transmission electron microscopy and/or epifluorescence microscopy) methods. DNA methods can involve either direct extraction of the viral sequences from soil or indirect extraction, with viruses firstly separated from soil followed by the extraction of sequences from the virus (Williamson et al., 2017).
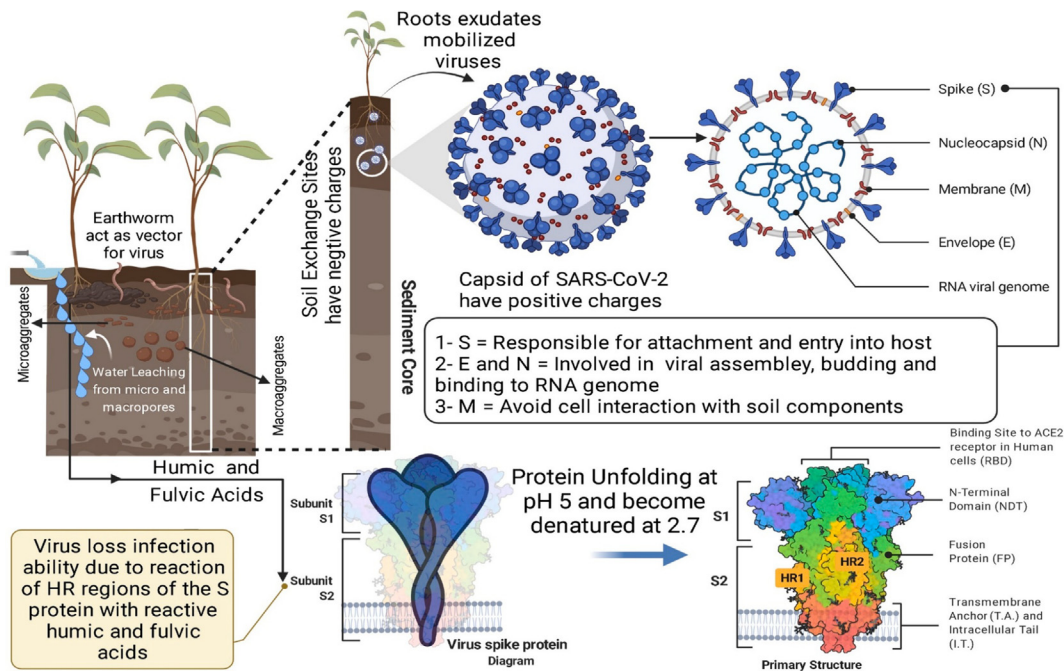
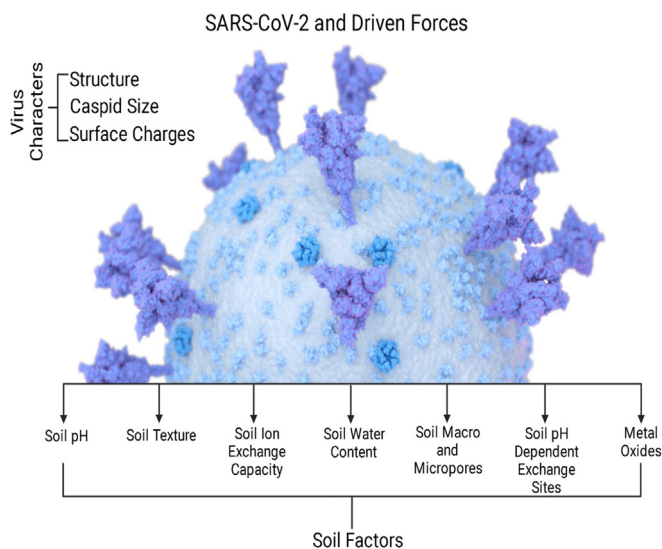**Fig. 1.** Hypothetical location of SARS-CoV-2 in soil.

Fig. 2. SARS-CoV-2 and their driven forces in soil.

The chemical and/or physical extraction methods are the main approach for direct and indirect viral sequences extraction from soil. The chemical approach permits the viruses elution from the soil by a series of sequential washes with increasing extraction force buffers to favor their desorption from the soil. The main limit is related to the uncertainty of the extraction buffers due to the extreme variability of the chemical-physical characteristics of the soils (Williamson et al., 2005, 2003). The main adopted extraction buffers are potassium citrate (1.44 g Na2PO4, 0.24 g KHPO4, pH7.0) (Williamson et al., 2011), sodium deoxycholate 0.1% (w/v) pH 7.0 (Williamson et al., 2011) or sterile deionized water, pH 7.0 (Ashelford et al., 2003). Pyrophosphate has also been largely used to extract viruses from sediments (Williamson et al., 2003). In a comparative study, the extractions with potassium citrate and sodium deoxycholate buffer seem to perform better in

sandy-silty and clay soil, respectively (Williamson et al., 2013). Related to the physical extraction approach, the main adopted methods are sonication, blending, bead-beating, and vortexing (Williamson et al., 2013). In the case of the combined approach, it has to consider the synergistic effects that may promote or inhibit the extraction efficiency (Williamson et al., 2013).

It is essential to underline that a robust extracting approach may lyse the capsid and damage the virus's nucleic acid. Further preserving viral RNA is important for producing high-quality sequence data by maintaining a cold chain between sample collection and sequencing. Once the viral nucleic acids are extracted, amplicon sequencing or metagenome analysis can detect viral sequences (Mokili et al., 2012). The target sequences of the SARS-CoV-2 are those encoding the spike one glycoprotein, that is, the viral protein exposed to the surface that allows the entry into host
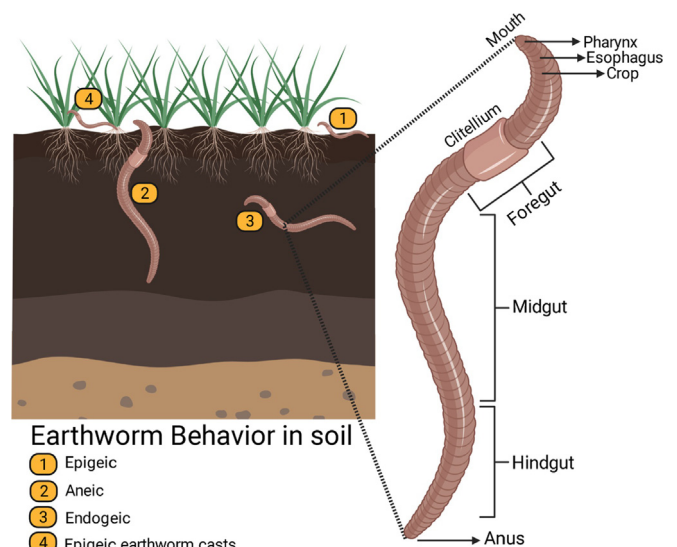

Fig. 3. Earthworms as virus accumulators in soil.

cells (Walls et al., 2020), and the consensus viral region RdRp of the pan β-CoV sequence (Zhou et al., 2020). Finally, a qPCR analysis should quantify the viral abundance. The qPCR analysis allows quantifying the viral abundance (Randazzo et al., 2020). Concerning the detection of the different SARS Cov-2 variants, different primer sets for amplicon-based sequencing have been tested with the target amplicons of different lengths, typically 400–2000 base pairs (bp) (Anantharajah et al., 2021). Finally, WHO has recently published a guide on Genomic sequencing of SARS-CoV-2 to provide technical advice to research groups (WHO, 2021).

We speculate that efficient extraction methods should involve repeated soil washings with solutions extracting viruses differently adsorbed to soil particles, with those loosely adsorbed being extracted before than those strongly adsorbed or bound. This extraction method may provide insights into virus mobility (bioavailability) and its potential spread, such as leaching water bodies. It is important to underline that strong extracting solutions may lyse the capsid and damage the virus's nucleic acid.

We can also speculate that the SARS-COV-2 can be extracted from soil using earthworms due to their ability to filter large quantities of soil and the presence of the slight acidic intestinal pH values (pH 6–6.5), which do not inactive the virus (Fig. 3) (Infante-Rodríguez et al., 2016). Earthworms are vectors of the foot-and-mouth disease virus that persist in earthworm bodies for up to 8 days in an active state (Edward and J.R., 1972). Aneic earthworms can collect viruses through the soil profile, whereas epigenic and endogenic earthworms can collect viruses from the surface and deep soil layers, respectively (Bouché, 1977). Once earthworms are isolated from soil, both the stomach and casts can be analyzed for the presence of viruses. Future research should evaluate the importance of this approach in determining the presence and the diffusion of the virus in the soil profile.

5. Conclusions and main research questions

Based on our knowledge of the possible distribution, behavior, and fate of SARS-CoV-2 in soil based on the virus capsid characteristics (well summarized in Figs. 1 and 2), we discuss the numerous unresolved questions that persist by identifying the following research to fill this knowledge gaps:

(i) Accurate analyses of the viral load of SARS-CoV-2 should concern irrigation waters, agrochemicals, fertilizers, pesticides, and edible parts of plants. The ways of virus entrance in plants need to be investigated as well as its persistence in plants and the movement to edible parts. Plant infection can occur not only by infection of the above-ground parts of the plant but also through wounds or cuts of roots and during the elongation phase of roots through the lack of connections between the emerging lateral root and the cortex of parent root (Esau, 1977). The importance of these studies goes beyond the simple food risk, highlighting how plants, although not target organisms of the virus, can host viruses and behave as vectors of diffusion. Another challenging task concerns the relationship between plants and SARS-CoV-2, focusing on the rhizosphere effect: do root exudates mobilize the virus from surface-reactive soil particles?

(ii) The setup of an accurate assay should start from what is already known about the determination of viruses in soil; that is, the direct determination of viral sequences is currently the preferred method (Pratama and van Elsas, 2019; Williamson et al., 2017). The method should concern the gene encoding the spike one glycoprotein and the consensus viral region RdRp of the pan β-CoV sequences. The qPCR quantification should accompany the analysis of sequences (Zhou et al., 2020). Noteworthy, the extraction yield of the viral sequences depends on the soil type because clay and organic soils require more efficient extracting solutions than sandy soils. However, the challenge is the compromise between getting an optimal extraction yield and avoiding the breakdown of the target viral sequences. Another challenge is the purification of the extracted viral nucleic acid to eliminate humic molecules or other soil components before qPCR analysis and amplicon sequencing;

(iii) Model studies as those carried out by Stotzky (Stotzky, 1986) are important for understanding the mechanisms underlying the adsorption of the virus to clay minerals, the maintenance of infectivity after the release of the virus from soil particles, the role of cations saturating negative charges of soil particles and the effect of other viruses and substances on the virus adsorption. These studies should also concern other surface-reactive soil particles and not only clay minerals. As reviewed by Kuzyakov and Mason-Jones (Kuzyakov and Mason-Jones, 2018), the role of viruses on soil microbial functions is poorly known. The SARS-CoV-2 is not a bacteriophage, and thus its effects on microbial functions are likely indirect; for example, competing with bacteria to adsorption sites. The location of the virus in the soil matrix is important for preserving its infectivity. Proteolysis of capsid proteins by proteases released by soil microorganisms is challenging if the virus adsorption involves these proteins. In addition, the virus localized in small micropores (<2 μm) may be inaccessible to bacteria, fungi, and extracellularly released proteases.

Some of the proposed research may use mutated strains to evaluate which parts of the virus are important in its spread and behavior in the plant-soil system. Noteworthy, the main detected mutation concerns the D614G protein that seems to increase the virus infectivity (Zhou et al., 2020). Models are important to predict soil functions, but only a few models focus on microbial functions, and none consider viral infection (Kuzyakov and Mason-Jones, 2018). However, models simulating infections rates, movement, and spatial localization in the soil of the SARS-CoV-2 should consider what known about other viruses in soil. In conclusion, researches on the origin, status, and behavior of SARS-CoV-2 are open and important. It is urgent to determine inputs, adsorption by surface reactive soil particles, maintenance of infectivity, and uptake by a plant of the virus in the plant-soil system, as discussed in this perspective. It is finally relevant to underline the appearance on the horizon of other possible viral threats capable to spillover on humans. The main one seems to be that the Swine Acute Diarrhea Syndrome Coronavirus (SADS-CoV) (Edwards et al., 2020) can potentially infect humans. The risk is also enforced by the wide world diffusion of the pork industry and also the swine manure utilization in agriculture with related frequent environmental pollution cases

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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