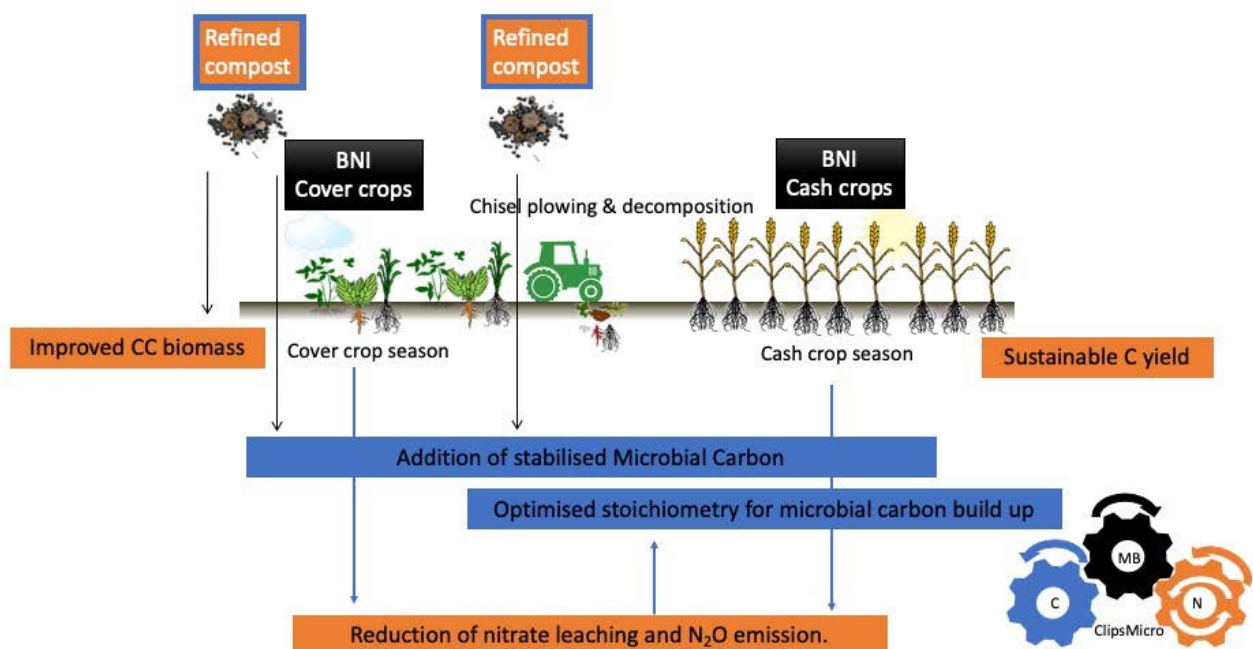# Exploring Cutting-Edge Strategies for Soil Carbon Enhancement and Emission Reduction

Mineral amended compost and crops with biological nitrification inhibition (BNI) capacity

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Preface

The **Cli**mate **p**roof **s**oils by steering soil and residue **micro**biomes (ClipsMicro) project is based on the central premise that soil microbes play a vital role in carbon sequestration and greenhouse gas emissions. The project aims to enhance stable soil carbon formation and reduce GHG emissions by effectively managing the soil microbiome. To achieve this, an integrated approach will be developed, involving improved soil amendments and strategic cover crop usage. Specifically, the project will focus on enhancing compost properties through biological and mineral amendments and integrating BNI (Biological Nitrification Inhibition) cover crops into cropping strategies. As part of the ClipsMicro project, this review explores the current state-of-the-art knowledge on the use of mineral additions in the composting process to increase organic matter sequestration and the application of biological nitrification inhibition in field conditions.

Content

Summary

To meet the goals of the Paris Climate Agreement and the European Union, innovative approaches on the increase of soil carbon stocks and mitigation of greenhouse gas emissions from the soil are needed. This review explores two strategies: (i) incorporating clay into the composting process to enhance mineral-associated organic matter formation, and (ii) cultivating crops that release biological nitrification inhibitors, which can modulate soil nitrogen cycling. The addition of clay to compost aims to improve carbon sequestration potential of sandy soils, while biological nitrification inhibitors can help mitigate the emission of the potent greenhouse gas nitrous oxide, reduce nitrate losses to water and increase nitrogen use efficiency by crops.

Incorporating clay into the composting process: Soil organisms play a crucial role in decomposing organic matter, and recent studies underscore the significance of microbes in stabilizing soil carbon. Importantly, organic materials, including the remnants of microbial activity, establish robust connections with soil minerals. These connections offer protection against decomposition. The specific surface area of the soil mineral matrix has a substantial influence on the soil's ability to forge these connections. Consequently, it profoundly affects the soil's capacity to preserve and protect soil organic matter (SOM). The interactions between organic matter and clay minerals involve multiple binding mechanisms, and different types of clay minerals vary in their ability to retain organic matter. While researchers have begun unraveling these interactions, the exact mechanism remains unknown. Nevertheless, given the pressing need to address climate change, incorporating clay minerals into the composting process holds promise as an approach to enhance SOM stabilization and increase carbon sequestration, particularly in sandy soils.

Cultivating crops that release biological nitrification inhibitors: Biological Nitrification Inhibition (BNI) holds promise in curbing N_2O emissions from agricultural soils. BNI is the attenuation of the nitrifying process resulting from compounds released in the soil from plant roots or turnover of plant tissue. Unlike synthetic alternatives, BNI compounds are natural alternatives for chemicals and can be continuously released by plants throughout the growing season. BNI compounds hinder nitrifiers through various mechanisms, such as enzyme inhibition and interference with bacterial quorum sensing. Plantain has shown potential as BNI crop in temperate regions, wheat and maize might be potential BNI crops in temperate regions and sorghum, signalgrass and rice have shown potential as BNI crops in (sub)tropical regions. Research is ongoing in screening and assessing temperate species which can produce BNI compounds. Until now, the majority of BNI research has taken place *ex-situ.* Field studies are essential to assess the impact of BNI crops on N cycling and N_2O emissions. Factors indirectly causing shifts in N cycling should be considered to disentangle direct BNI effects, such as changes in soil characteristics (e.g. pH or water filled pore space), (historical) N loading, microbial N immobilization and competition for N between microbes and plants.

Knowledge gaps: Knowledge gaps related to soil organic matter (SOM) formation and its relation to soil mineralogy were identified. Firstly, the relative contribution of microbial and plant sources to the mineral-associated organic matter (MAOM) remains uncertain. Investigating the interactions between plants, microbes, and carbon incorporation into this stable SOM pool is crucial for effective composting practices that increase the stable SOM concentration. Secondly, there is still an insufficient understanding of the interplay between soil mineral composition and the retention of organic matter. Although the presence of sporadic and clustered three-dimensional (3D) mineral-bound organic matter (MAOM) has been observed, obtaining a more holistic comprehension of how microscale and nanoscale soil and organic matter structures synergistically function could significantly improve the precision of predicting the effects caused by specific soil components. Grasping this dynamic relationship between organic matter and minerals holds the potential to refine composting techniques through the incorporation of mineral amendments. Thirdly, despite the existence of several studies investigating the effects of introducing minerals to compost for organic matter sequestration, none of these studies have specifically differentiated between mineral-associated organic matter (MAOM) and particulate organic matter (POM). Last, a research gap lies in the exploration of how the introduction of minerals to compost might alter its fertilization capabilities. Exploring these issues is crucial for making informed decisions on optimal composting practices and for effectively harnessing compost as a sustainable fertilization source.

Similarly, there is a strong need to study the potential of BNI crops in practice. It is highly recommended for this project to prioritize studying the BNI potential of crops in a field context and to focus less on the increase of fundamental BNI mechanisms, since field studies are scarce and environmental and biological interaction effects can significantly change the BNI potential. Herein, it is recommended to integrate a diversity of field management practices and fields with different soil textures in the experimental design. Studying the link between BNI crop root physiology, rhizodeposition and microbial N-cycling could help to define crop breeding priorities for BNI. At the same time, aspects such as crop establishment, persistence and management under field conditions deserve attention to understand the potential of BNI crops during their growing season as well as after (cover) crops have been incorporated in the soil.

Overall, the reviewed strategies of refining the composting process with clay minerals and utilizing (cover)crops with biological nitrification inhibitors holds potential for enhancing soil carbon sequestration and mitigating greenhouse gas emissions in agricultural systems. Although further investigation is required to gain a comprehensive understanding of their mechanisms and efficacy within practical, uncontrolled settings, these strategies present innovative avenues to confront the pressing challenges of climate change.

1 Introduction

Global soils contain more organic matter than vegetation and the atmosphere combined (Ciais et al., 2014; Eswaran et al., 1993). Nearly half of the Earth's soil surface is utilized for agriculture, which leads to the release of carbon dioxide (CO_2), methane, and nitrous oxide emissions (Paustian et al., 2016). Reversing the decrease in soil organic matter (SOM) through amendments could mitigate climate change and enhance soil health (Hoffland et al., 2020). However, the nutrient balance in these sources amendments might not match microbial needs, leading to increased decomposition of old organic matter when nutrients are lacking or increased nitrogen/phosphorus release when carbon is scarce. Both these pathways can result in more nitrite/nitrate production, potentially leading to nitrate leaching and greenhouse gas emissions, such as N_2O. Balancing increased carbon storage with reduced nitrous oxide and methane emissions is a challenge. To address this challenge, climate-smart agricultural practices have been developed to enhance carbon sequestration and improve soil quality while reducing greenhouse gas (GHG) emissions (Paustian et al., 2016). These practices are vital to meet Paris Climate Agreement and European Union emission reduction goals, maximizing agricultural soil's climate change mitigation potential.

Composting is a traditional method for converting organic residues into a stable soil improver that can increase the soil organic carbon content when added to soil (Barthod et al., 2018) However, it is crucial to consider the nutrient stoichiometry, specifically the carbon-to-nitrogen-to-phosphorus ratio of compost, as they may not align with the requirements of soil microbes. This disparity can potentially stimulate the decomposition of existing soil organic matter, known as the priming effect, in situations where nitrogen or phosphorus is limited. Alternatively, it can lead to increased nitrogen or phosphorus mineralization when carbon is limited. As a result, nitrification can occur, resulting in elevated nitrite/nitrate production (Lazcano et al., 2021). The increased production of nitrite/nitrate may lead to nitrate leaching from the soil or the release of the potent greenhouse gas, nitrous oxide, at nitrification and denitrification (Norton & Ouyang, 2019). This greenhouse gas production has the potential to reduce or even negate the climate benefits gained from carbon sequestration.

To align with the objectives of the Paris Climate Agreement and the European Union, it is imperative to increase soil carbon stocks while mitigating greenhouse gas emissions. This review highlights two innovative approaches: incorporating clay into the composting process to enhance the formation of mineral-associated organic matter (carbon), and cultivating cover crops that release biological nitrification inhibitors to counteract nitrogen-related greenhouse gas emissions. The incorporation of clay during composting aims to enhance the resilience and potential for sequestration of organic carbon in the soil. The adoption of cover crops brings a dual benefit: in addition to releasing biological nitrification inhibitors, it also contributes to increased carbon sequestration through the root exudation of carbon

compounds during growth, as well as through ploughing them under at the beginning of spring. The implementation of both these methodologies holds the promise of advancing sustainable soil management practices, significantly contributing to climate change mitigation.

1.1 Aim of the literature review

The aim of this literature review is to investigate innovative approaches to simultaneously increase soil carbon stocks and mitigate greenhouse gas emissions. Specifically, the review focuses on two strategies: (1) the incorporation of clay into the composting process to enhance the formation of mineral-associated organic matter, and (2) the cultivation of (cover)crops that release biological nitrification inhibitors to counteract nitrogen-related greenhouse gas emissions and improve carbon sequestration at the same time. By synthesizing existing literature, this review aims to provide insights into the potential effectiveness of these methods and contribute to the development of sustainable soil management practices for climate change mitigation.

2 Literature part A: soil organic matter and composting

In part A of the literature review will focus on the topic of incorporating clay into the composting process to enhance the formation of mineral-associated organic matter

2.1 Redefining soil organic matter: microbes and mineral interactions

Carbon in soils is present in the form of inorganic C (carbonite minerals) or as part of soil organic matter (SOM), the latter accounting for the majority of soil C (950 Gt SOM vs. 1550 Gt total C; Lal, 2004). Carbon that is present within SOM is referred to as soil organic carbon (SOC), and the term is often used interchangeably with SOM. The proportion of SOC in SOM is highly variable but most often ranges between 40-71 % (Pribyl, 2010).

Organic matter enters the soil via plants (in the form of decomposed plant roots, root exudates, and aboveground plant debris) and animals (through their droppings and the remains of both deceased animals and soil-dwelling microorganisms). Soil organisms, upon the entry of organic matter into the soil, play a crucial role in its decomposition by consuming it (Frouz, 2018). Larger soil organisms, such as earthworms, fragment and mix the organic matter by passing it through their digestive systems, and combine the undigested portions with soil and microorganisms (Sokol et al., 2019). Part of the organic matter is consumed, either by larger macro-/meso-organisms, or micro-organisms, and becomes part of the organism's own biomass. Another proportion is respired during metabolism and leaves the soil as carbon

dioxide (CO_2) or methane (CH_4). The remaining organic matter has now been broken down into smaller organic molecules, and may be subjected to further breakdown and consumption by soil (micro)organisms (Cotrufo et al., 2013).

In the last decade a paradigm shift surrounding the fate of SOM and the formation of stable soil carbon has occurred (Lavallee et al., 2020a; Lehmann & Kleber, 2015). In the "old" paradigm, the general thought was that the stabile pool consisted largely of very recalcitrant plant derived organic molecules that microorganism could not break down (further), and could reside in the soil for tens to hundreds of years. However, an increasing body of evidence indicates that the stable organic carbon reservoirs within the soil derive not only from plant biomass, but also significantly from deceased microbial biomass, commonly referred to as microbial necromass (Liang et al., 2019). This suggests that the stabilization of organic matter can also occur following the decomposition of plant material, and the stabilization of Soil Organic Matter (SOM) occurs during its cycling rather than primarily through its recalcitrance (Lehmann & Kleber, 2015). A recently proposed and now widely applied model divides carbon in two pools, based on the mechanism by which they are protected from decomposition (Lavallee et al., 2020b): the mineral associated organic matter (MAOM) pool and.the particular organic matter (POM) pool

The MAOM pool is protected from decomposition via strong connections to soil reactive surfaces (e.g. clay minerals, iron (hydr)oxides, and is therefore considered to be the long-term stable sink for soil carbon. A considerable portion of MAOM is derived from the in-vivo microbial breakdown of plant litter, followed by the absorption to minerals of microbial necromass and metabolic by-products generated during the decomposition process (Liang et al., 2019). Another pathway leading to MAOM formation involves the adsorption of dissolved water soluble polar plant compounds onto soil minerals. These two pathways of MAOM formation favor different sources of organic matter: the microbial pathway is chiefly regulated by substrates characterized by high microbial carbon use efficiency. These substrates are labile and possess a high nitrogen to carbon ratio. On the other hand, the direct sorption pathway is primarily associated with organic matter that possesses physicochemical attributes conducive to robust interactions with soil minerals, notably attributes like polarity (Sokol et al., 2019).

The POM pool is characterized by its susceptibility to decomposition, and is primarily retained in the soil due to its chemical resistance against microbial breakdown. The POM pool primarily consists of plant molecules that are biochemically recalcitrant in the short term, making them less accessible to microbial utilization, and unable to directly bind to soil minerals. Although the organic compounds within the POM pool exhibit short-term recalcitrance, they eventually undergo mineralization and be respired (Schmidt et al., 2011) or incorporated in microbial biomass. The speed of decomposition of free POM is strongly influenced by soil environmental

factors, such as pH or oxygen levels, that affect microbial activity. Sometimes POM is physically protected from decomposition trough inclusion within soil aggregates. When POM is enclosed in small micro-aggregates (<50 μm), its turnover time is similar to that of the mineral-associated organic matter (MOAM). However, protection within larger aggregates does not significantly enhance the long-term stability of enclosed POM compared to freely available POM (Lavallee et al., 2020b).

2.1.1 The way forward

Since the formation and behavior of MAOM and POM in the soil are distinctively different, it is essential to assess these fractions separately. In order to gain a comprehensive understanding of the fraction of effectively stabilized organic soil amendments, it is essential to assess the formation and behavior of MAOM and POM fractions separately as their behavior in the soil is distinctively different. Additionally, considering the significant role of microbes in MAOM formation, changes in microbial biomass and microbial community composition under different amendment strategies might also offer valuable indications regarding soil organic matter dynamics.

2.2 Unveiling the impact of clay minerals on soil Organic matter dynamics

There is clearly an interaction between organic matter and minerals. For example, the clay-size fraction (<2 um) of a soil is a good predictor for total SOM stocks (e.g. Hassink, 1997; Rakhsh et al., 2017; Wiesmeier et al., 2019; Xiang et al., 2022), and adding clay to a soil can increase soil carbon concentrations (Churchman et al., 2020). The ability of soil to retain and protect SOM is often found to be strongly related to the specific surface area of a soil (e.g., Ndzana et al., 2022; Singh et al., 2017; Xu et al., 2022). The surface area is controlled by a combination of the particle size distribution (primarily clay and fine silt contents), clay mineralogy, organic matter (OM) content, and iron and aluminum oxide contents (Kaiser & Guggenberger, 2003). Nevertheless, results are not always consistent, and direct evidence relating OM storage to mineral surface is scarce (e.g. Schweizer et al., 2021; Zacháry et al., 2023). In order to progress our understanding of MAOM formation, it is important to consider and unravel the mechanisms by which organic matter reacts with soil minerals. This chapter aims to provide a current overview of the state-of-the-art research on the mechanisms underlying the interaction between organic matter and soil minerals.

2.2.1 Phyllosilicates and metallic oxides: key players in soil organic matter stabilization

Phyllosilicates (clay minerals) and metallic (hydr)oxides have been identified as the two types of primary clay-sized minerals that most significantly impact the stabilization of soil organic matter (Churchman et al., 2020). In most soils, phyllosilicates are the majority of the fine-grained fractions, whereas metal oxides are a magnitude less abundant (Kleber et al., 2021).

Phyllosilicates are aluminosilicate minerals with a sheet-like structure in which sheets are connected via intersheet bonds. They can be classified into two types based on their structure, namely 1:1 and 2:1 phyllosillicates (Figure 1). In addition to these main structural types, there are also modulated phyllosilicates that exhibit a nonstandard structure. These modulated phyllosilicates are commonly found in volcanic soils and exhibit unique characteristics compared to the regular phyllosilicates. The 1:1 structure, seen in minerals like kaolinite, consists of a single tetrahedral sheet and one octahedral sheet. These sheets stack on top of each other with strong bonds to form a layer. The 2:1 structure, found in minerals like smectite and vermiculite, consists of two tetrahedral sheets sandwiching an octahedral sheet. Isomorphic substitution of Si_4^+ by Al_3^+ in tetrahedrons and Al_3^+ by trivalent or bivalent atoms in octahedrons leads to a net negative charge. This substitution occurs more frequently in 2:1 clay layers. Both 2:1 and 1:1 clay layers exhibit charges that vary with pH due to hydroxyl groups on the exposed edges of the clay layers. Because of the more negative charge in 2:1 silicates compared to 1:1 silicates, they typically have a greater carbon exchange capacity (CEC) (Barré et al., 2014).

Phyllosilicates occur in soils as stacks of several layers of the same or different identity. The interlayer bonding between different layers further determines clay characteristics. Interlayer bonds are relatively weak compared to covalent intersheet bonds. Kaolin minerals with 1:1 layers have interlayer bonding based on hydrogen bonds, while mica-type minerals like illite (2:1) layers are connected via anhydrous molecules like potassium. Smectites layers are connected through weak Van der Waals bonds, enabling the interlayer space to be filled with water which cause the clays to swell. Water expandable phyllosilicates have a larger specific surface area than non-expendable sillicates (800 m2g-1 vs. 10-70 m2g-1) (Wiesmeier
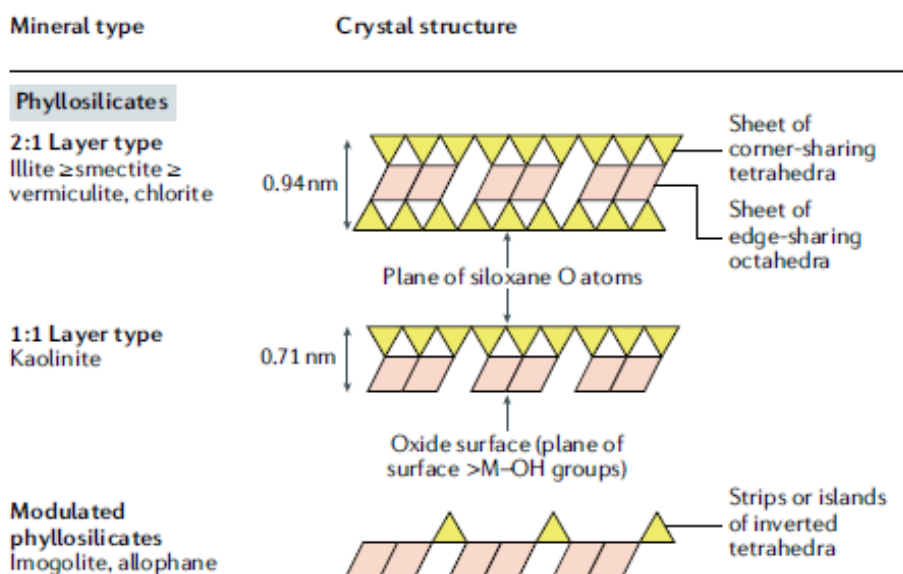

Figure 1: Crystal structure of phyllosilicates from Kleber et al., 2021

et al., 2019). Generally, weaker interlayer bonding leads to a smaller clay particle size and, therefore, increased specific surface area. Furthermore, short-range-order aluminosilicates such as allophane and imogolite have a large surface area due to their lack of a consistent crystal structure (Wiesmeier et al., 2019). When metal hydroxides intercalate the interlayer space of 2:1-type minerals, hydroxyl-interlayered clay minerals are formed. For example, Al hydroxides can exist in the interlayer space of vermiculite (Liu et al., 2023).

Metal oxides have an even greater affinity for SOM than phyllosilicates (Wiesmeier et al., 2019). The presence of metal oxides, especially Al- and Fe-oxides, has a significant effect on the specific surface area. Removal of these metal oxides has been found to cause a substantial reduction in surface area (Jiang et al., 2014). A recent study found a positive correlation between the Fe- to aluminous clay ratio and the MAOM fraction (Kirsten et al., 2021), suggesting that not only the individual components but also mineralogical combinations determine organic matter stabilization. The maximum sorption capacities of these metal oxides have been found to occur within pH ranges of 4.3-4.7, where ligand exchange between OH functional groups and carboxyl/phenolic OH functional groups is optimal (Singh et al., 2018).

2.2.2 Organo-mineral interactions

Multiple binding mechanisms play a role in the interaction between organic matter and clay minerals. These mechanisms are ligand exchange, polyvalent cation bridging, electrostatic attraction and hydrophobic weak interactions including H-bonding and van der Waals forces (Singh et al., 2017). Among these interactions, the most powerful one is ligand exchange, which specifically occurs in acid soils where protonated hydroxyl groups are present. Cation bridging happens when a positively charged ion connects to both a negatively charged clay and OM, creating an indirect organo-mineral interaction. Further, electrostatic interaction involves cation exchange, in which a cation that is connected to mineral surface gets replaced by a positively charged (section of an) organic matter molecule. Further, weak interactions occur through induced dipole interaction (van der Waals force) or attraction of partial charges (H-bonding). Additionally, interlayer hydroxyl-Al, which typically is a gibbsite like structure (complex of Al(OH)3) can attract and stabilize soil organic matter in the clay-interlayer. This form of organic matter stabilization has been found in one experiment to account for 24% of the total mineral-associated organic matter and be highly temperature stable and resistant to oxidation (Liu et al., 2023).

Clay minerals exhibit varying capacities for retaining soil organic matter, with different phyllosilicates showing different effectiveness. Allophane is widely recognized as the most proficient in SOM storage, followed by smectite, illite, and kaolinite (Bruun et al., 2010; Rakhsh & Golchin, 2018). The contrast between 2:1 and 1:1 clays in binding soil organic matter is a commonly observed phenomenon (e.g. Six et al., 2002). This difference is often attributed to

the larger surface area of 2:1 clays, although surface area isn't always the sole determinant in SOM stabilization. For example, D'acqui et al. (1998) discovered that the decomposition of organic matter was more pronounced in montmorillonite (2:1) soils compared to kaolinite (1:1) soils, after 30 days of incubation at as 25 degrees Celsius, kaolinite clay contained more residual C as compared to montmorillonite. Moreover, Vogel et al. (2015) observed that illite-based (2:1) artificial soils accumulated more organic matter in the mineral fraction than montmorillonite-based (2:1) soils, despite the latter's greater surface area. The illite soil showed higher microbial biomass and subsequent mineralization compared to the montmorillonite soil. The authors proposed that this led to a greater sequestration of microbial products in the clay-sized fraction. A similar mechanism may have been at work in the D'acqui et al. (1998) study. Furthermore, Rasmussen et al. (2018), analyzed data from over 5500 soil profiles across continental scale environmental gradients and found clay content to have relatively little explanatory power compared to other parameters, namely exchangeable calcium for alkaline soils and iron- and aluminum-oxyhydroxide for more acid soils. Lastly, metal oxides and short-range order (SRO) minerals, known for their elevated surface area and surface charge, generally display a greater capacity for OM sorption than phyllosilicate clays, which have been found for both wheat and eucalyptus derived inputs (Sanderman et al., 2014).

2.2.3 Revisiting the paradigm: dynamic perspectives on mineral-associated organic matter

Since the change in perception of SOM stabilization and the recognition of the mineral associated C-fraction as an important C sink, the mechanisms that guide this association have been studied more intensely.

Vogel et al. (2014) looked at OM distribution on clay minerals using SEM (scanning electron microscopy). Surprisingly, only 13-26% of the minerals appeared as individual particles with mostly plain surfaces, while the majority (74-87%) existed in clusters of several small particles with rough surfaces, exhibiting etch pitch, micropores, and cracks. The distribution of OM was found to be heterogeneous, occurring in small patches preferentially on the mineral clusters. Only 1-2% of the individual mineral surface was covered with organic matter whereas 15-21% of the mineral clusters was covered. Consequently, approximately 19% of the mineral surface was associated with organic matter, contradicting the common belief that the entire clay surface is capable of forming mineral-associated organic matter. Furthermore, additional OM was preferentially attached to organo-mineral clusters with rough surfaces that already contained pre-existing OM, leading to no increase in total area covered with OM. Accordingly, in a study by Schweizer et al. (2021),the capacity of soils, with a clay gradient ranging from 5% to 37%, to retain stable organic matter was not significantly different along the gradient. Surprisingly, in soils with a relatively low clay content (ranging from 5% to 18%), the fine mineral-associated organic fractions exhibited up to double the organic carbon (OC) content compared to soils with high clay content.

The recent advancements in research have led Schweizer (2022) to propose a novel conceptual framework of organo-mineral associations. According to this framework, mineral-associated organic matter (MAOM) is primarily concentrated within heterogeneous patches of organic matter OM rather than being distributed evenly across the entire surface area, and subsequent OM-OM interactions contribute to the vertical accumulation of organic matter (see also Possinger et al., 2020). If this is true, this means that the abundance of mineral surface area alone may not dictate the capacity for storing organic carbon. Instead, the establishment of OM-OM interactions within localized areas may play a crucial role, potentially creating a protective mechanism that is vertically layered and extends in three dimensions. Schweizer further suggests that the heterogeneity resulting from patchy distribution and vertical accumulation of OM could elucidate the divergent findings observed in various studies concerning the effect of mineralogy on SOM dynamics.

2.3   The mineral puzzle: practical strategy for enhancing organic matter retention

As demonstrated in previous section, the intricate interaction between soil minerals and SOM remains a prominent area of ongoing research. We acknowledge and recommend these research endeavors for their potential to enhance our understanding, predictive capabilities, and manipulative techniques concerning global SOM sequestration. However, given the urgent nature of climate change, we cannot afford to await a complete resolution of the mineral puzzle. Timely action is imperative, as climate change progresses irrespective of scientific certainties. The complex interplay between organic matter and soil minerals underscores the significance of the total surface area of soil minerals as a relatively accurate indicator of SOM sequestration potential. This realization has sparked innovative ideas to increase carbon sequestration: ClipsMicro proposes the incorporation of clay into the composting process as a means to enhance SOM stabilization in sandy soils with a limited intrinsic SOM storage potential and minimize C losses through mineralization.

2.3.1   Compost

In agricultural soils, carbon dynamics are influenced by a wide array of management decisions, including crop selection (with varying amounts of crop residues), fertilizer choices (mineral or organic), and the extent of soil tillage. The use of compost can increase the SOM concentration and by doing so improve its related ecosystem functionalities such as soil fertility, soil structure and water holding capacity (Adugna, 2016; Diacono & Montemurro, 2011; Hoffland et al., 2020). Compost is produced by piling up organic material and letting it decompose by microorganisms under controlled, aerobic conditions and temperatures up to 70 degrees (Barthod et al., 2018; See box 1). The resulting compost is free from weed seeds and pathogens, making it safe to use in agriculture. Additionally, it is easy to spread on the land and contains valuable organic matter and plant nutrients that enrich the soil.

Box 1. The composting process

The composting process can be divided into three main phases based on temperature and microbial activity: initial, thermophilic, and maturation - phase. Based on Barthod et al., (2018).

The **initial activation phase** is characterized by low temperatures and relatively slow microbial activity. During this phase, mesophilic bacteria and fungi begin to break down simple sugars, starches, and soluble proteins into smaller compounds such as organic acids, alcohols, and carbon dioxide. This phase typically lasts for a few days, in which the temperature of the pile increases in a linear way.

The **thermophilic phase** is the most active and important stage in the composting process. During this phase, the temperature of the pile reaches a maximum due to the metabolic activity of thermophilic microorganisms. These microorganisms are capable of breaking down complex organic compounds such as cellulose and lignin into simpler molecules such as humic acids and amino acids. The temperature in the pile can reach up to 60-70°C which can last for several weeks to a few months. The high temperatures achieved in the compost pile have been shown to effectively remove pathogenic microorganisms as well as inactivate fungi (Insam and de Bertoldi, 2007).

The **maturation phase** is the final stage of the composting process. During this phase, the temperature of the pile decreases as the microbial activity slows down from a decrease of biodegradable compounds. The compost is stabilized. The mature phase can last for several weeks to a few months, depending on the type of feedstock used and the composting conditions. The compost is matured when turning the pile does not result in its reheating.

Composting involves the use of various feedstock, ranging from plant materials such as leaves and grass clippings to animal waste and food scraps, depending on the intended use of the final product. Each feedstock has a different nutrient content and decomposition rate, which can affect the quality of the final compost and the extent of GHG emissions. Emission of CH_4 occurs during the anaerobic microbial decomposition of organic matter, and is positively correlated with OM concentration and temperature (Zhou et al., 2022). During the thermophilic phase of composting, the conditions for CH_4 emissions can arise as a consequence of heightened microbial activity, leading to elevated temperatures and the development of localized anaerobic environments (Guo et al., 2021). The initial stage of composition is also susceptible to CH_4 emissions due to the high moisture content on low availability of oxygen. The CH_4 emission

is mitigated by a high concentration of NH_4^+ ions, usually the case in acidic environments, that results in the inhibition of methanogens. Emissions of CO_2 are mainly due to microbial respiration during aerobic decomposition of organic matter (Liu et al., 2020). Therefore, CO_2 emissions are highest during phases with high microbial activity which is indicated by higher compost temperatures (Huang et al., 2022).

Composting is beneficial for soil health and offers a sustainable alternative to landfilling and incineration. However, it's crucial to address the carbon emissions—CO_2, CH_4, and N_2O—associated with composting (Huang et al., 2022). Recent studies have emphasized that composting can lead to the loss of over 40% of carbon, primarily in the form of CO_2 (Ye et al., 2023). To mitigate this carbon loss, an intriguing strategy involves incorporating mineral (clay) particles during composting. This addition has the potential to enhance the integration of microbial necromass and metabolic by-products into the stable MAOM pool. As a result, the resulting clay-enriched compost could significantly enhance the soil's capacity for carbon sequestration when applied to the soil.

Considering this approach raises several pertinent questions. Should the assertion that the inclusion of minerals during composting indeed leads to an increased formation of Mineral-MAOM prove accurate—a matter demanding in-depth investigation—it could suggest the necessity for tailored composting conditions to optimize this process. For example, since MAOM predominantly originates from microbial decomposition byproducts, necromass, and labile dissolved plant compounds, the choice of composting materials and conditions that are in alignment with these considerations, encompassing rapid decomposition, low carbon-to-nitrogen ratios of feedstock, becomes advantageous. Furthermore, this approach underscores the vital importance of comprehending the intricate interplay between organic matter and minerals. Such understanding is pivotal to selecting the most optimal mineral amendment for the composting process. Additionally, the improvement of microbial anabolism and, consequently, carbon use efficiency during composting could enhance organic matter sequestration. Unfortunately, limited scientific studies have explored this issue. For comprehensive reviews on the role of microbes in composting/inoculation, readers are referred to Wei et al. (2022) and Oviedo-Icaña et al (2022).

Notably, conditions conducive to MAOM formation are conducive to rapid decomposition. Consequently, the overall composting processing time might significantly decrease due to this accelerated decomposition, potentially leading to elevated heat generation and, in turn, a decrease in total carbon emissions. However, this effect requires meticulous investigation to establish its validity.

2.3.2 Enhancing composting with mineral additives

To our knowledge, there are no studies that address the question of specifically MAOM formation in compost heaps. However, the addition of (clay) minerals to the composting process is not new. An article by Barthod et al (2018) provides an overview of various additives (biological and synthetic), and their impacts on the composting process. Regarding the mineral additives, they are primarily utilized to mitigate odors, reduce greenhouse gas emissions, minimize nutrient leaching, and enhance water retention in compost.

The addition of clay minerals can influence both the composting process as well as compost properties. For instance, the inclusion of different clay minerals has been found to prolong the thermophilic phase of composting, subsequently shortening the overall composting duration, which is economically advantageous (Himanen & Hänninen, 2009; Wu et al., 2023). Similarly, Venglovsky et al., (2005) observed rapid temperature increases when zeolite was introduced during the composting of solid fraction pig manure. Conversely, the addition of bentonite did not alter the temperature profile in the composting of a pig manure and corn stalk mixture (R. Li et al., 2012). Furthermore, addition of minerals may impact the pH throughout the composting process, which might influence emissions. Typically, pH decreases during the initial stages, and increases in the later stages (Onwosi et al., 2017). Zeolite, due to its ammonia adsorption properties, has been found to counteract the pH increase during the thermophilic phase of composting (Venglovsky et al., 2005). However, it is worth noting that the addition of zeolite can negatively affect the soluble phosphorus content in compost, as it may contribute to the formation of insoluble phosphorus compounds or complexes, although this is not necessarily a problem as compost is not considered a P fertilizer. Moreover, several studies have reported that the addition of clay during composting contributes to the immobilization of heavy metals, resulting in a reduction of their toxicity (Wu et al., 2023; Zheng et al., 2022).

The current scientific literature on the effects of mineral addition in the composting process on the stabilization of organic matter or carbon emissions specifically, remains limited. However, a few studies have provided insights into this subject. Barhold et al. (2016) discovered that the inclusion of montmorillonite clay mineral at 25% or 50% (w/w) in pre-composted material resulted in lower CO_2 emissions during composting. As argued by the authors, this may indicate carbon stabilization by physicochemical protection of OM on clay surfaces. Further, in a recent study conducted by Wu et al. (2023), the impact of incorporating 5% (v/v) of sepiolite and palygorskite, both individually and in combination, was investigated during the composting of chicken manure to assess their effect on humification. After a composting period of 30 days, both the combined and independent clay treatments exhibited a significantly higher total organic carbon content compared to the control treatment without clay addition (31% vs. 35–50%). Similarly, Zeng et al. (2022) observed that the addition of sepiolite during pig manure composting facilitated compost maturation and

increased the percentage of humic acids by 2.8–10.7%, accompanied by an increase in aromaticity. Pan et al. (2021) reported that adding 7.5% (w/w) of montmorillonite or illite during chicken manure composting resulted in decreased OM reduction. In the control treatment, the initial OM content (70%) decreased by 29.4%, while in the montmorillonite and illite groups, the reductions were 29.1% and 28.9% respectively. Further, adding diatomite, a kind of porous silicate material, to (manure) composting has been shown to reduce CH_4 emission and increase CO_2 emissions and dissolved organic carbon decomposition (Ren et al., 2019). A 10% addition of diatomite, based on dry weight, was observed to be optimal for reducing CH_4 emissions and greenhouse gas emission equivalents (Ren et al., 2021). Medina et al. (2020) examined the impact of adding either iron oxide or allophane-rich (25% w/w of allophane) soil to the composting process at a rate of 2% (w/w) on organic matter stabilization. They found that the addition of allophane-rich soil had no effect on the carbon content (C%) of the final product. However, the addition of iron oxide had a very slight but positive effect, increasing the carbon content to 44% compared to 43% with no addition and allophone-rich soil addition. However, it should be noted that the higher C content observed with the addition of iron oxide does not necessarily indicate greater carbon storage, as the total biomass loss during composting with iron oxide may have been higher compared to the other conditions. Unfortunately, total biomass loss was not analyzed by the authors. Furthermore, although not clay minerals, addition of superphosphate, superphosphate + ferrous sulfate, or a mixture of all three to compost, can reduce CH_4 emissions by 50.6-73.6%, which was explained by effects of the additives on methanogens (Peng et al., 2019). Methanotrophs might also be effected by these compounds (van den Bergh et al., 2023), but in the current study only two methanotrophs appeared in the compost at low concentration and therefore did not cause this effect. In artificial soil (so not compost), an addition of clay resulted in higher microbial biomass and similarly higher organic matter concentrations (Rakhsh et al., 2017). However, high imogolite concentrations resulted in lower microbial biomass, but still increased carbon sequestration, arguably due to the fact that the strong covalent interactions were limiting the availability of OM for soil microbes, resulting in no decomposition but similarly no microbial growth (Rakhsh et al., 2020). The same effects might be observed when imogolite is added during composting, but this remains to be tested.

In the previously mentioned studies, the compost had varying compositions with clay content spanning from 5 to 50% (w/w). Deciphering the optimal quantity of clay to enhance the composting procedure for OM sequestration most likely hinges on numerous variables, notably the composting conditions and the particular clay variant(s) selected. Considering the innovative nature of augmenting OM stability by adding minerals to the composting process, the precise and most effective ratio of clay to minerals remains unresolved. It is important to exercise caution with clay addition. An excessive incorporation of clay may yield undesirable outcomes. For instance, it could impede aeration, diminishing the availability of oxygen to organic matter and consequently promoting anaerobic digestion. Additionally,

compost must by Dutch law conform to certain standards. Importantly, a minimum of 10% of the dry weight should consist of organic matter. While this percentage might seem modest, it's worth noting that organic matter holds a substantial water content, whereas the introduced minerals are devoid of moisture.

3 Literature part B: BNI and nitrogen emissions

Organic and synthetic nitrogen (N) inputs are essential for crop growth. Part of the N in compost and organic manure comes available through mineralization of organic matter. The ammonium-N ($N-NH_4^+$) that becomes available is taken up by plants and immobilized by microbes to sustain their growth. Another part is converted to other forms of N, like nitrate (NO_3^-) that can leach to deeper soil layers and groundwater in case it is not taken up by plants or immobilized. Within this microbial mediated N-cycle, nitrous oxide (N_2O) can be formed, which is 298 times stronger GHG than CO_2. Some cover and main crops show BNI activity, which could attenuate the nitrification of NH_4^+ to NO_3^-, and could therewith potentially reduce N_2O emissions from both nitrification and denitrification. Before diving deeper in the BNI concept, we will provide some more detail on the N cycle and N_2O formation to support understanding of the mechanisms at work.

3.1 Formation of nitrous oxide in the soil

3.1.1 Nitrification and denitrification

Under both aerobic and anaerobic soil conditions, soil N mineralization takes place through decomposition of soil organic matter. The resulting available N in the form of NH_4^+ can be immobilized by plants and soil micro-organisms to sustain their growth. Another part of NH_4^+ is nitrified by soil microbes: it is converted to NO_3^- via the intermediates hydroxylamine and nitrite. During nitrification, the N containing compounds are used as electron donors and are oxidized for energy supply. Although multiple microbial groups contribute to the nitrification process, they are often summarized as ammonium oxidizing bacteria (AOB) and archaea (AOA) and nitrite oxidizing bacteria (NOB). AOA and AOB contribute mainly to the first two steps of nitrification (from NH_4^+ to NO_2^-) and NOB to the third step of nitrification, which is the oxidation of NO_2^- (Nardi et al., 2020) (Figure 2). For this process, they mainly use CO_2 (AOB, NOB) or HCO_3^- (AOA) as carbon source (Nardi et al., 2020). AOA are better adapted to oxygen poor conditions than AOB, while AOB can better handle acid soil conditions.

Complete ammonia oxidizers (comammox) have only recently been discovered. They are able to oxidize both NH_3 and NO_2^- within the same cell completely to NO_3^- (Prosser et al., 2020). Because of their high NH_3 affinity, comammox, like AOA, flourish better than AOB under NH_4^+ limited conditions, and comammox are therefore suggested to produce lower amounts

of N_2O compared to AOB. AOB generally play a more important role in environments with high NH_4^+ input, due to their competitive advantage at higher soil NH_4^+ concentrations (Prosser et al., 2020).

During the oxidation steps in the nitrification process, gaseous losses of N_2O can take place, and NO_3^- can leach to deeper soil layers (Figure 2). NO_3^- can be converted through a process called denitrification: this is again a cluster of reactions in which NO_3^- is converted to N_2 via NO_2^-, NO and N_2O. Although this is merely a dominant process under anoxic conditions, it could also prevail under oxic circumstances by aerobic denitrifiers and in soil aggregates or under conditions of high oxygen consumption. In this microbial process, N containing compounds are functioning as oxidators; they accept electrons and use OM compounds as source of electrons (Figure 2). Note lots of denitrifiers are facultative anaerobes and they can easily switch between using oxygen and nitrate as electron acceptor.

3.1.2   Formation of N_2O during (de)nitrification

During the process of nitrification as well as denitrification, N_2O can be formed, which is a strong greenhouse gas. Nitrification rates and therewith N_2O formation rates, depend on multiple environmental factors (Figure 3). Nitrification rates increase with temperature, oxygen and NH_4^+ content and decreasing pH. The highest N_2O production at nitrification is found at sub-optimal conditions, e.g. during wet (more anoxic) conditions (Velthof & Rietra, 2018). The fraction of N_2O emitted instead of converted to N_2 during denitrification, i.e. N_2O yield, generally increases with increasing NO_3^-, oxygen and organic carbon concentrations and with decreasing temperature and pH. It is difficult to relate N_2O emissions to either nitrification or denitrification; a decreased nitrification, so less nitrate formation, will also affect N_2O emission rates during the denitrification process. Furthermore, potential large emissions of N_2O can also be generated during another process, nitrifier denitrification, but not much is known about this pathway of N_2O production (Velthof & Rietra, 2018). This pathway may play a dominant role under oxygen limitations or variable oxygen concentrations (Wrage-Mönnig et al., 2018).

No evidence for nitrifier denitrification is found in AOA and comammox bacteria, since NO reductase genes are lacking in the bacteria (Prosser et al., 2020). Lower N_2O yield of AOA and conammox compared to AOB are therewith suggested and reported (Prosser et al., 2020).
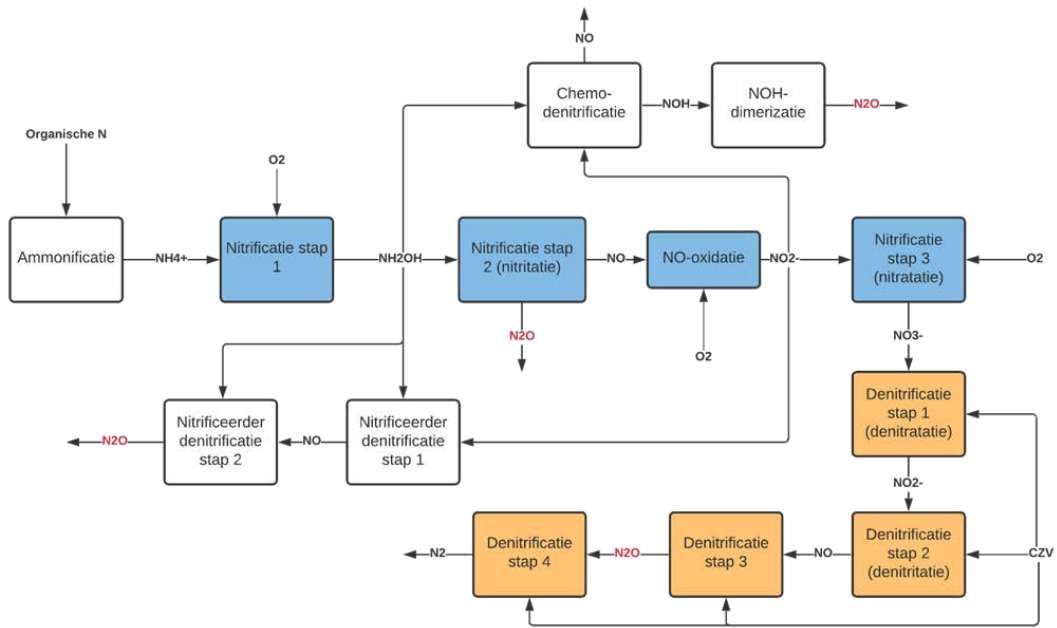
Figure 2: Nitrogen cycling and potential nitrous oxide formation sites (De Heer, 2021, unpublished.
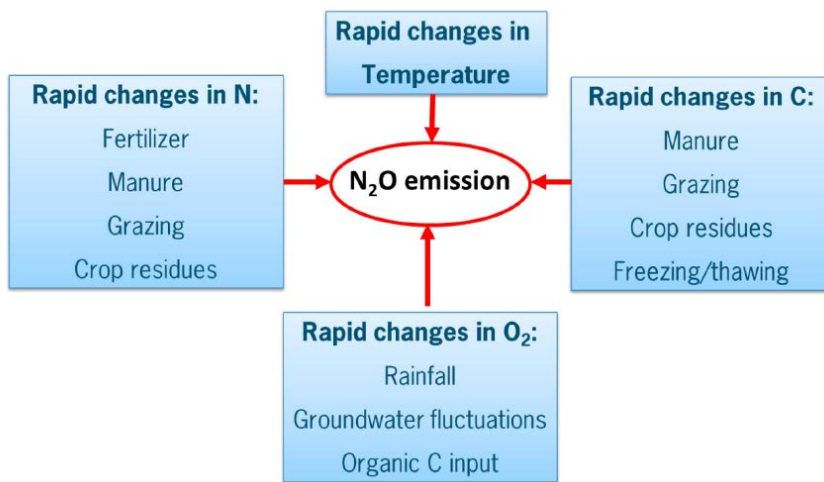
Figure 3: Environmental factors influencing formation of N_2O (Velthof & Rietra, 2018)

3.2   BNI and N_2O emissions

BNI (biological nitrification inhibition) is a promising pathway for reducing N_2O emissions from agricultural soils. BNI is the attenuation of the nitrifying process resulting from compounds released in the soil from plant roots or turnover of plant tissue (De Klein et al., 2022). BNI may reduce N_2O emissions and leaching of NO_3^- via the attenuation of the nitrifying process.

Although synthetic nitrification inhibitors (SNIs) provide the possibility to reduce N_2O emissions by application at specific times, locations and doses, BNIs also have their advantages: 1) they

can reach the nitrifying sites of the soil because they are directly released in the root zone; 2) they affect both the ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO), two enzymes active in nitrification in micro-organisms, while SNIs only affect the AMO pathway; 3) synthetic production and mechanical application are not needed; 4) BNIs could be continuously provided by plants throughout the growing season and 5) BNIs are a nature-based solution, creating space for higher public acceptance, while the use of SNIs is subject to regulations and their use may not always be allowed (Nardi et al., 2020).

Compounds with suspected BNI capacity are found within the chemical groups of phenolics, fatty alcohols and acids, terpenes, isothiocyanates and amino acids (Nardi et al., 2020). Most of them are found within the group of polyphenolics, which are known for inhibiting N mineralization and increasing N immobilization (e.g. (Fierer et al., 2001; Schimel et al., 1996). Low molecular weight phenolics are more likely to be used as a substrate by microbes (Fierer et al., 2001), so it is especially the heavier molecules that could potentially inhibit nitrification.

The effectiveness of BNIs highly depend on their release rates from crop tissue incorporated in the soil or through rhizodeposition and their residence time before degradation (Nardi et al., 2020). The distribution through the soil layer also depends on the chemical structure of the compounds; hydrophilic compounds will have a better distribution through the soil solution in the rhizosphere, while hydrophobic compounds will target the area directly in the vicinity of the active root zone. This is because of the higher absorbance of hydrophobic compounds to mineral and organic surfaces (Subbarao et al., 2013, 2015). Relative contributions of hydrophobic and –phyllic compounds probably highly vary with plant species and amongst growth stages. For example, in sorghum, hydrophobic BNI compounds play a larger role in the early growth stages, while hydrophilic compounds show increased relative impact at later growth stages (Subbarao et al., 2015).

3.2.1   Direct BNI mechanism: targeting microbial nitrifiers

BNI compounds in rhizodeposits or from decaying plant tissue can affect microbial nitrifiers in the soil, and thereby nitrification and N_2O emissions, via different mechanisms being : 1) outer membrane permeabilization; 2) nitric oxide binding; 3) quorum sensing inhibition and 4) AMO and HAO enzyme inhibition. Acting as outer membrane permeabilizers and targeting cytoplasmatic membranes, BNIs can paralyze ammonium oxidizing bacteria (AOB) and archaea (AOA) (Nardi et al., 2020). Certain BNI compounds, e.g. quercetin and caffeic acid, could furthermore bind nitric oxide (NO) ("NO scavengers"), inhibiting further nitrification. NO is an intermediate form in the nitrification process, and is essential for NH_3 oxidation by AOA and AOB. BNI compounds can also inhibit bacterial quorum sensing activity, which is a communication process essential for the production of enzymes involved in the degradation of low molecular weight organic-N compounds, and via this pathway nitrification may also be attenuated (Nardi et al., 2020).

The fourth BNI mechanism of enzyme inhibition is the most widely studied. Different authors have demonstrated that certain BNI compounds could directly affect microbial nitrifier activity by inhibiting the AMO or HAO enzyme, or both at the same time (Byrnes et al., 2017; Coskun et al., 2017; Gao et al., 2022; Lu et al., 2019; Sarr et al., 2020; Subbarao et al., 2009, 2013; Sun et al., 2016). Subbarao et al. (2015) provided an overview for the relative effectiveness of BNI compounds in bioassays, and which nitrification enzymes they inhibit. The authors postulated that both AMO and HAO were inhibited when the NH_2OH substrate was not degraded anymore after addition of a BNI compound (Subbarao et al., 2006, 2013). This method is not suitable anymore, since it was recently suggested that NO is the intermediate between NH_2OH and NO_2^-, and that even a third enzyme could play a role in NO_2^- formation (Nardi et al., 2020). Therefore, BNI compounds could not only act on AMO and HAO, but also on this third, unidentified enzyme.

A direct effect of BNI compounds on nitrification is often identified by quantification of AOA and AOB populations and amoA gene abundance (Kaur-Bhambra et al., 2021; Lu et al., 2019, 2022; Subbarao et al., 2009; Sun et al., 2016)(Nardi et al., 2020). It is just recently found that also AOA can play an important role in N_2O formation, although it is not clear what their relative role is in this sense under field conditions (De Klein et al., 2022). AOA flourish better than AOB under N poor (Byrnes et al., 2017; De Klein et al., 2022; Trivedi et al., 2019) and oxygen poor conditions (De Klein et al., 2022; Ke et al., 2013) and seem to be stronger competitors for (labile) C inputs than AOB (Ai et al., 2013; Ke et al., 2013). In some studies, effects of BNI compounds on AOA *amoA*-gene abundance is demonstrated, while no or a less strong relation with AOB was found (Byrnes et al., 2017; Kaur-Bhambra et al., 2021; Sarr et al., 2020). While in others studies the BNI compounds seem to affect both AOA and AOB activity (Lu et al., 2019, 2022). Although we will not further dive into the effect of BNI compounds on denitrifiers, Li et al. (2022) found in an experiment with *Elymus* grasses with different BNI activity that reduced N_2O emissions positively correlated with *nirS* gene expression of denitrifiers.

It should be reflected upon that a plethora of methods are used to analyse dynamics of microbial populations and activity, all with their pros and cons. Nardi et al. (2020) provides a good overview of current microscopic methods that can be used next to PCR techniques to analyse the effect of BNI compounds on soil microbial dynamics. Molecular, ex-situ techniques have the disadvantage that microbial dynamics cannot be studied within their soil micro-environment, and result of ex-situ measurements are difficult to translate to practical conditions. However, sssthese culture-based techniques can provide valuable information on the direct effects of BNI compounds on microbial groups that can support microscopic and PCR-based in-situ observations (Nardi et al., 2020).

For claiming a direct effect of BNI compounds on AO populations and therewith nitrification, it is important to perform more field experiments. Putative effects of BNI compounds have been especially tested in bioassays and soil-assays. While effects were shown in bio-assays, these effects didn't always translate to soil-assays (Lu et al., 2019). It is unclear how long BNI compounds from growing plants or died-off plant material stay active after release in the soil environment.

3.2.2 Nitrification rates indirectly influenced by plants

Nitrification can also indirectly be adapted by plants shaping their rhizosphere and beyond (Bowatte et al., 2016). One should therefore be careful in making claims about direct legacy effect of BNI compounds when only measuring potential nitrification rates in bioassays or NO_3^-/NH_4^+ ratios in soils and leachate with growing plants (Bowatte et al., 2016). There are many reasons that make it difficult to attribute shifts in N cycling directly to rhizodeposition of BNI compounds (Bowatte et al., 2016; Nardi et al., 2020):

- one plant species or cultivar could simply create more extended root systems and grow faster, immobilizing more N by more uptake, which make it essential to take overall crop growth into account when determining inter- and intraspecific BNI effects on nitrification;

- plants could alter microbial ammonia oxidizing communities and activity by competition for N (Ai et al., 2013; Ke et al., 2013; Kuzyakov & Xu, 2013; Ma et al., 2020; Trivedi et al., 2019) and indirectly by altering their soil environment (e.g. pH, soil moisture, temperature, C/N ratio) (Ai et al., 2013; Trivedi et al., 2019);

- plant C inputs (including LMW BNI rhizodeposits) changing soil C/N ratios could cause microbial communities to shift towards more N immobilization, causing a drop in nitrification activity (Fierer et al., 2001; Nardi et al., 2020; Schimel et al., 1996);

- one cultivar may have a higher affinity for NH_4^+ compared to NO_3^- than the other (Sun et al., 2016);

- high NH_4^+ concentrations may inhibit nitrite oxidizing bacteria, resulting in less NO_3^- production (Nardi et al., 2020);

- in the case soil C is not limiting, heterotrophs may outcompete slow growing nitrifiers for NH_4^+, because of their higher growth rate and NH_4^+ affinity, maintaining a high demand for N. Altogether this could cause shifts to more N immobilization, resulting in less nitrification not directly affected by BNI compounds (Nardi et al., 2020);

- soils high in expansible clay minerals (amorphous iron oxides) and SOM, resulting in high exchange surfaces, could further immobilize NH_4^+, indirectly affecting N cycling (Nardi et al., 2020);

- regarding AOA, BNI polyphenolic compounds could also indirectly cause nitrification inhibition by acting as a copper chelator. Unlike AOB, AOA are dependent on copper in an electron transfer mechanism essential for NH_4^+ oxidation (Lu et al., 2019).

3.3 Current status: which crops and exudates have BNI potential?

Besides all complexities regarding directly demonstrating BNI legacy effects on N cycling, there are different crops showing potential for inhibiting nitrification and thereby N_2O emissions. Rhizodeposits with compounds showing BNI activity have especially been found amongst (sub)tropical crops (De Klein et al., 2022). For sorghum (*Sorghum bicolor*), signalgrass (*Brachiaria humidicola*) and rice (*Oryza sativa*) rhizodeposits identified as BNI compounds have been described (Coskun et al., 2017; Subbarao et al., 2021; Zhang et al., 2022a). Regarding sorghum, these are sorgoleone, sakuranetin and methyl propionate; regarding signalgrass, these are brachialactones; and regarding rice, decanediol is found as a BNI active compound (Zhang et al., 2022a). These compounds are found to directly target microbial nitrification by affecting AMO and HAO microbial enzyme activity (Coskun et al., 2017).

For these crops, research is still ongoing to understand the BNI mechanism, its side-effects on crop parameters, and how nitrification inhibition is related to the cultivar and environmental context. In a greenhouse experiment, Sarr et al. (2020) demonstrated that sorgoleone rhizodeposition has great potential to inhibit AOA abundance and soil nitrification. Gao et al. (2022) found higher hydrophilic BNI activity and thereby nitrification inhibition and reduced N_2O emissions in two high compared to two low sorgoleone producing sorghum cultivars. This was possibly related to reduced nitrifier populations (AOA, AOB) in the rhizosphere.

Both a higher inhibition of soil nitrifier activity and inhibition of N_2O production from urine patches were found in a signal grass cultivar with high BNI capacity compared to one with low BNI capacity (Byrnes et al., 2017). It should be noted that the high BNI cultivar had a higher root biomass, thus a probably more extended root system, which may have contributed to lower soil NH_4^+ concentrations and N_2O emissions (Byrnes et al., 2017). Longer term studies are therefore needed to predict the impact of high BNI activity signal grass on microbial populations and functions, and N_2O emissions.

Progress has also been made in demonstrating BNI activity of 1,9-decanediol (Lu et al., 2019) and syringic acid (Lu et al., 2022) from rice. These compounds negatively affect nitrifier communities and N_2O emissions, and in the case of 1,9-decanediol specifically inhibit the AMO pathway for ammonia oxidation (Sun et al., 2016). Effects were more pronounced in acidic soils and less in calcareous ones (Lu et al., 2019, 2022). Nitrification inhibition was found to strongly correlate with NH_4^+ uptake and NH_4^+ relative to NO_3^- uptake, indicating that promising BNI-rice varieties could also improve nitrogen use efficiency (Sun et al., 2016). It should be noted that the BNI compounds from rice were only applied to soil in incubation experiments in high concentrations, indicating that field studies exploring the potential of high BNI active rice varieties is still needed.

For wheat (*Triticum aestivum*) and maize (*Zea mays*) there are indications for BNI active compounds (Coskun et al., 2017; Subbarao et al., 2021). A screening study with 96 wheat landraces (O'Sullivan et al., 2016) selected 26 varieties of which extracts and exudates showed inhibition of nitrification by ammonia oxidizers. Exudates of four varieties showed nitrification inhibition in a sandy loam soil medium. It is unclear if the applied exudates were actively secreted or a result of root mass turnover. All in all, potential is shown for breeding BNI characteristics in wheat landraces into elite wheat cultivars. It has been shown that transferring the BNI potential in elite wheat cultivars through traditional breeding approaches with 'synthetic hexaploid wheats' that have not been subjected to any selection pressure in high nitrifying soil environments has potential (Subbarao et al., 2015). In another study, an arm of chromosome 3B of a high yielding wheat cultivar was replaced by an arm wheat grass (*Leymus racemosus*) controlling BNI production, resulting in a doubling of BNI production and retained yield potential (Subbarao et al., 2021). This case showed the potential of BNI active chromosome transfer for nitrifier inhibition, reduced N_2O emissions, reduced levels of soil NO_3^- plants shifting to NH_4^+ uptake strategy and faster SOM mineralization (Subbarao et al., 2021).

Regarding maize, hydrophobic compounds zeanone, HDMBOA, HMBOA and HDMBOA-beta-glucoside, with putative BNI activity, active in the direct root vicinity, have been isolated and identified (Otaka et al., 2022). These would count for respectively 19%, 20%, 2% and 4% of hydrophobic BNI activity in maize roots. Their real effect on N cycling in the soil matrix from lab to field scale needs to be further identified, including their impact on N_2O emissions.

Strongly reduced N_2O emissions have been found with increased plantain (*Plantago lanceolata*) cover in dairy grassland on peat soil (Pijlman et al., 2020) and with field applications of aucubin extracted from plantain (Gardiner et al., 2018). However, no direct interaction was identified between nitrification inhibition and BNI compounds in rhizodeposits in these studies.

A mesocosm study on urine-affected Elymus grass cultivars showed an interaction effect between cultivar and N_2O emissions, indicating potential for targeted selection on this trait (W. Li et al., 2022). The authors suggested that the diversity in N_2O emissions between cultivars could be partly explained by denitrifier activity: relations between reduced N_2O emissions and lower expression of *nirS* gene-bearing bacteria (denitrifiers) were found (W. Li et al., 2022). This putative biological denitrification inhibition (BDI) effect on N_2O emissions has been suggested in other studies where plant extracts or specific BDI compound procyanidins from *Fallopia* spp. were applied to soil under controlled (Bardon et al., 2014)and field conditions (Galland et al., 2019). However, for these cases a direct effect of BDI or BNI compounds in rhizodeposits on N_2O emissions need to be tested under field conditions.

As mentioned, current studies on BNI crops on ammonia oxidizing populations and activity have often been executed under controlled conditions and also for some crops only by directly applying specific rhizodeposits with BNI compounds in bioassays with certain pre-selected microbial populations. Under field conditions, results will very likely differ for multiple reasons: 1) more rhizodeposits can work synergistically as nitrification inhibitors, 2) BNI compounds are probably released in pulses with different concentrations rather than constant supply and 3) probably released in lower concentrations than in part of the published bioassay results (Lu et al., 2019). Furthermore, BNI effectiveness will depend on 4) the soil volume penetrated by the active root system, 5) the transport of hydrophilic compounds to different soil compartments outside of the root vicinity, where ammonia oxidizing communities are also active (Ai et al., 2013; Ke et al., 2013; Trivedi et al., 2019) and the local soil microbial community which could also consume LMW BNI compounds instead of being inhibited by them (Fierer et al., 2001). Finally, BNI releases from plants may also depend on the soil chemical composition and available nutrient to plants, since different nutrients can stimulate plants to produce different exudates.

3.4   The effects of BNI on N cycling under field conditions

The assumed advantage of cultivating BNI crops over using SNIs for nitrification inhibition is that BNI crops could provide nitrification inhibition compounds through the whole season. In controlled experiments, BNI compounds have been found to be able to induce nitrification inhibition months after their release (Gopalakrishnan et al., 2009; Sarr et al., 2020; Subbarao et al., 2013, 2015). For hypothesizing BNI effects on N cycling under field conditions however, two main factors should be considered, which will be discussed in the following sections: 1) environmental factors have an effect on BNI compound rhizodeposition, distribution and effectiveness; 2) plant age and developmental stage affect the amount and concentration of BNI compounds in rhizodeposits.

3.4.1   How are BNI rhizodeposition and distribution in the soil affected by environmental factors?

Where many edaphic factors could quickly affect N_2O emissions in soils (Figure 3), similar factors could also affect rhizodeposition, distribution, stability and effectiveness of BNI compounds on the shorter and longer term.

First of all, BNI rhizodeposition depends on the $N-NH_4^+$ availability in soils. In a split root experiment it was shown that the exudation of the hydrophobic sorgoleone BNI compound was more positively affected by NH_4^+ than by NO_3^- in a solution (Zhu et al., 2012). Furthermore, Sarr et al. (2020) found for sorghum plants a positive relation between the release of sorgoleone in the soil and $N-NH_4^+$ supply. Also Zeng et al. (2016) confirmed a direct effect of NH_4^+ assimilation on the sorgoleone BNI release, in an experiment where the root exudate collection solution was kept constantly at pH 5.

Rhizodeposition and retention time of BNI compounds in soils is highly affected by soil pH (Subbarao et al., 2007). That is why it should be noted that NH_4^+ applications acidify the soil and thereby indirectly affects BNI compound release (Zhang et al., 2022b). It has been shown for signal grass, sorghum as well as rice that an low pH (below xx) stimulated BNI release, while at pH above 7 BNI release was reduced from signal grass and sorghum (Zhang et al., 2022b). An low soil pH is thus expected to promote BNI release, which could inhibit nitrification causing accumulation of NH_4^+ in soils. This will promote further acidification, creating a positive feedback for BNI release (Di et al., 2018; Gao et al., 2022; Subbarao et al., 2007; Zhang et al., 2022b).

Soil moisture conditions further dictate the release and distribution of rhizodeposits including BNI compounds. Moderate soil moisture conditions (mesic conditions), neither being deprived from oxygen nor being dry, are ideal situations for root growth and thereby BNI release and distribution through the soil volume (Zhang et al., 2022b). Dry soil conditions (xeric conditions) could cause BNI compounds to accumulate in the root vicinity, hinder root growth and thereby further BNI release; high soil moisture conditions (hydric conditions) are also not stimulating for root growth and BNI release of terrestrial plants (Zhang et al., 2022b). On the other hand, in a study with different pearl millet cultivars, rhizodeposition of BNI compounds per gram root dry weight seemed to increase under drought stress for one cultivar. This was suggested to be related to an observed increased root length under drought stress as well as to N deficiency (Ghatak et al., 2022). It is plant species and variety dependent if drought stress and nutrient deficiencies lead to root system expansion and an increased release of secondary metabolites initially, or that the root system activity is reduced.

To study the direct effect of soil moisture on BNI activity is very difficult, for example because soil moisture also influences the growth and activity of the ammonia oxidizing community and thereby the nitrification rates (Sarr et al., 2020). Immobilization of ammonium by reactive mineral surfaces and SOM may also interact with the BNI effectiveness after being released. Although some work has been done on concentrations of secondary metabolites in plants under elevated temperatures and CO_2 conditions (Jamloki et al., 2021), it is not clear yet what this means for BNI release from plants.

3.4.2 How is BNI rhizodeposition and distribution influenced by growth stage and cultivar?

It is important to know at what phase of development the BNI potential of crops is the highest, in order to exploit their contribution to nitrification inhibition in agriculture (Sun et al., 2016). Tissue concentrations and the release of BNI compounds have been shown to depend on plant development in different studies. Sun et al. (2016) observed six-week-old rice seedlings to have more BNI activity then three-week-old seedlings under controlled conditions, however

opposite results with higher activities at younger age have also been found (Pariasca Tanaka et al., 2010). Between 40 and 130 days after seeding, total BNI activity from sorghum root tissue was shown to increase, but when expressed per gram dry weight root, it decreased (Subbarao et al., 2013). Finally, Fuchs and Bowers (2004) observed higher concentration of the BNI compound aucubin in plantain leaves after 11 weeks of development compared to 6 weeks of development. However, they did not explore if there was a relation between the leaf concentrations and root exudation of this BNI compound. Studies with sorghum also showed more hydrophobic BNI compound release during early development (first two weeks) while after 30 days, there was no difference anymore in the release of hydrophobic and hydrophilic compounds (Subbarao et al., 2015).

Ke et al. (2013) observed growing AOA populations in the rhizosphere during rice development. Sarr et al. (2020) also confirmed this by their finding of changes in AOA and AOB *amoA* gene abundance during sorghum development. The growth of AOA populations could indicate changing BNI rhizodeposition patterns during plant development and a changing BNI activity.

As already shown, the rhizodeposition rates of BNI compounds is both species and cultivar dependent. Also, the distribution of BNI compounds through the soil volume is species and cultivar dependent: it depends on root system architecture, but also on the ratio between hydrophilic and hydropobic compounds released. Effectiveness of BNI rhizodeposits released could also be species and cultivar dependent, because each one of them affects their own rhizosphere microbial community amongst others through the amount of labile LMW C released, their role in SOM decomposition and the extent in which they influence rhizosphere pH. Variation may also be determined by the extent to which microbial communities consume BNI compounds, rather than being inhibited by them.

3.4.3 BNI and the current application of cover crops and crop rotations

The number of field experiments carried out that applied a systems approach to study the effect of BNI crops on nitrification and N_2O emissions is minimal. In a recent review a significant effect of cover crops, without proven BNI activity, on N_2O emission reduction in humid but not in drier climates was found (Ekwunife et al., 2022). Since the cover crops taken into account in this review had no proven BNI effect, their effect in humid climates on N_2O emission could be the of competition for N resources. Furthermore, the reviewers only took winter emissions into account and this does not reflect the overall effect of cover crops on emission reductions. Winter emissions accounted for 5% to 91% of the annual N_2O emissions (Ekwunife et al., 2022). Preza-Fontes et al. (2020) found strong N_2O reductions when sorghum varieties (*Sorghum bicolor* x *sorghum bicolor* var. Sudanese) were used as a cover crop. However, this effect was compromised by higher emissions in the following growing season. This could have been caused by quick fertilization after the crop was planted and C inputs after soil incorporation

of the cover crop, which promotes denitrification (Ekwunife et al., 2022; Preza-Fontes et al., 2020). This again stresses the importance of applying a system approach for studying BNI (cover) crop potential for N_2O emission reduction including different agricultural management regimes. In this same review (Ekwunife et al., 2022), described that clear N_2O reductions were found for no-tillage systems compared to tillage. This no-tillage effect was stronger at drier climates and coarse textured soils than at humid climates and medium textured soils (Ekwunife et al., 2022).

Different studies on intercropping and rotations with BNI crops have been performed recently. In a study with a 3-year maize-signal grass intercropping system, the effect of BNI crops on nitrification and N_2O emissions was minimal since cumulative N_2O emissions increased in the intercropping systems compared to a maize monocropping system (Canisares et al., 2021). The BNI effect of signal grass could have been outweighed by the observed effect of signal grass on soil moisture, N recycling and microbial community affecting soil N_2O emissions (Canisares et al., 2021). A field study with a BNI-sorghum and wheat rotation showed an increase in cumulative N_2O emissions compared to a fallow-wheat rotation, although *amoA* gene expression was reduced (Bozal-Leorri et al., 2023). It was hypothesized that this could have been caused by sorghum stubbles left in the field and no-tillage, causing peaking heterothropic denitrification fed by high C input. Recently, reduced peaks of N_2O emissions were observed a few days after fertilization in a 1.5 month established sorghum-maize intercrop system compared to maize monocrop system. This phenomenon was directly linked to $N-NH_4^+$ and $N-NO_3^-$ availability, AOA and AOB *amoA* gene expression and BNI compounds (Zhang et al., 2023). However, cumulative reductions over the whole growing season were not considered.

3.4.4   BNI crops in the Dutch agricultural practice and policy context

The use of a cover crops in maize cultivation on sandy soils is obligatory in the Netherlands, because the nutrient uptake of maize ceases during the growing season while organic matter mineralisation continuous. A cover crop is either sown directly or shortly after the sowing of maize, i.e. underseeding, or is sown after the harvest of maize. Currently allowed species of cover crops that can be underseeded or seeded directly after the harvest of maize are fodder rape (*Brassica napus*), fodder radish (*Raphanus sativus* var. *oleiferus*), grass, oat grass (*Avena strigosa*), triticale (×*Triticosecale*), winter rye (*Secale cereale*), winter wheat (*Triticum aestivum*), winter barley (*Hordeum vulgare*) and spelt (*Triticum spelta*). The cover crop may be mown or shredded and ploughed at the 1st of February as earliest, provided that it had been sown before the 1st of October. When the cover crop has been sown after the 1st of October, it has to be used as a cash crop in the following growing season, meaning that it cannot be ploughed in spring. In that case only triticale, winter rye, winter wheat, winter barley and spelt are allowed as cover crop. Mixtures of cover crops are allowed at the condition that two-thirds of the mixtures consists of the allowed species and that other species come to

development in autumn (RVO, 2022). This means that within current regulations there is limited space for BNI crops outside of the allowed species.

Fodder maize is typically harvested between half September and half October for optimal fodder quality. Maize is typically grown in rotation with grass leys at dairy farms on sandy soil, and the majority of the farms do not cultivate cereals as feed but cultivates grass after three years of maize cultivation or cultivates maize continuously. Since cover crops may only be destroyed in spring if they are sown before the 1st of October, many dairy farmers harvest their maize before the 1st of October. This means that cover crops are typically sown in the last week of September. Cover crops after the cultivation of maize are typically mulched and rotavated or shallowly ploughed in early spring (February-April) when soil conditions are favourable and before the crop has become too large. Cover crops producing a large amount of biomass in spring will also take up more nutrient and water from the soil, which can negatively affect the following crop. Nutrients immobilised in the cover crop have first to be mineralised before these are available for the follow up crop. Thus, BNI species as cover crop should come to development and have effects on N_2O emissions between October and February, and are preferably crops that do not produce large amounts of biomass in early spring. Cover crop mulch or residues left in the field could however possibly have a legacy effect on nitrification and thereby N_2O emission reduction by N immobilization and BNI compound release during its decomposition.

4 Knowledge gaps based on review topics A and B

The ideas of incorporating clay during composting to enhance SOM content and utilizing BNI producing crops to reduce nitrous oxide and nitrate emissions are both relatively new and, therefore, considered innovative. However, the novelty of these approaches gives rise to various unknowns, which we define as "knowledge gaps." In this chapter, we will explore the most significant knowledge gaps associated with these innovative strategies and provide corresponding recommendations.

Gap 1: Organic matter formation

In the last two decades, the traditional view of soil organic matter formation as a result of chemically recalcitrant plant molecules has shifted to an understanding of organic matter formation as a product of microbial decomposition of litter. Labile plant molecules are more efficiently decomposed by microbes, resulting in more microbial necromass, which can subsequently be very efficiently stabilized through association with minerals. This led to the belief that most of the MAOM (mineral-associated organic matter) was microbial-derived. However, in the last few years, this view has been challenged again, as it has been found that in some soils a significant fraction of MAOM is plant-derived. This is important because both

pathways of MAOM formation (microbial-derived versus plant-derived) select for very different litter qualities. Gaining a better and context-dependent understanding of how and when MAOM is formed will aid in improving SOM management practices. In the context of ClipsMicro, it could help in selecting the right compost constituents (e.g., labile versus recalcitrant materials).

Recommendation: It is recommended to utilize isotopic labeling techniques to trace the journey of labeled organic substances through the composting process and their subsequent migration into the soil. This approach is instrumental in assessing their assimilation into distinct organic matter pools both during composting and later when the compost is amended to soil. The beauty of this method lies in its ability to uniquely label different plant structural categories, shedding light on their individual pathways and whether these compounds ultimately find their place within the MAOM or POM pools within compost and later in the soil matrix. Consequently, this knowledge can guide recommendations on the most suitable organic matter sources for fostering stable MAOM formation. The exploration of potential interactions between different clay types and composting materials adds another layer of complexity. Experimenting with varying combinations of clay types and initial compost constituents can yield valuable insights into optimizing compost-clay interactions for enhanced SOM sequestration and overall soil health.

Going beyond the immediate scope of ClipsMicro, there's an intriguing avenue for fundamentally exploring the mechanisms and driving forces behind SOM cycling. In particular, delving deeper into the role of microorganisms in MAOM formation could yield insights that inform the judicious selection of compost constituents and optimal composting conditions. This deeper understanding could subsequently be harnessed to optimize the entire process for more effective SOM management. An efficient approach to address this would involve discerning the origin (whether from plants or microorganisms) of the mineral-associated organic matter (MAOM) in correlation with the structural characteristics of its organic matter compound.

Gap 2: Distinguishing POM and MAOM and their origin in compost

While there have been limited studies quantifying the effects of adding clay during composting on SOM stabilization in the compost, a critical gap exists as the distinction between MAOM and POM has not been explored in these studies. MAOM, being more stable and less susceptible to decomposition, plays a crucial role in sequestering carbon over extended periods, contributing to long-term carbon storage. In contrast, POM is more labile and prone to rapid decomposition, leading to relatively short-term carbon retention. Hence, making this distinction is essential when estimating the compost's potential for long-term carbon sequestration.

Recommendation: when investigating the effects of adding clay minerals during composting on organic matter sequestration in the compost, do make a distinction between POM and MAOM and dive deeper into the conditions needed for optimal MAOM formation during the composting process.

Gap 3: Soil mineralogy vs SOM sequestration:

The exact relationship between soil mineralogy and organic matter is not fully understood. Different indicators, such as total clay content and mineral surface area have often been found to correlate with SOM content. Nevertheless, conflicting results have been obtained. Recently, a new framework has emerged in which the organo-mineral complex is regarded as a dynamic and 3D interaction between numerous clay constituents with organic matter, occurring only at specific mineral regions. In order to optimize the SOM content in fields which received clay mineral amended compost, insight in these interactions is crucial.

Recommendations: To advance both scientific comprehension and practical advancements, two recommended approaches emerge. The first, while not directly aligned with the purpose of clipsMICRO, stands as essential work to be undertaken. Given the unpredictable nature of soil-mineral interactions, a critical imperative is to delve deeper into the intricate complexation between organic and mineral components at a foundational level. This pursuit will yield valuable insights into the inherent potential of global soils to sequester SOM, as well as the appropriate measures for accomplishing this task. Advanced multidimensional imaging techniques, such as computed tomography, can provide access to crucial soil parameters including pore size, pore morphology, and pore connectivity. By juxtaposing these soil attributes with the constituents of the soil matrix and concentrations of organic matter, a heightened comprehension of their intricate interplay can be achieved. This, in turn, paves the way for the formulation of precise strategies and adept management practices aimed at optimizing SOM sequestration and elevating the soil's capacity for carbon storage.

While fundamental insights into soil-mineral interactions are crucial, the climate agreement targets are that strict that we cannot wait until all interactions are fully understood. Hence, the second recommendation underscores the practical approach of employing the specific mineral surface area, currently regarded as the most accurate indicator, as a proxy for the potential organic matter concentration. Consequently, adding mineral(surface) during composting is likely to increase the soil organic matter concentration in fields at which the clay enriched compost is spread, primarily in the form of stable MAOM. We recommend testing this hypothesis in a field experiment on sandy soil used for maize cultivation. Furthermore, the amount of clay that should be added for the optimal effect is largely unknown. More is not necessarily better, as negative effects can be expected beyond a

certain point, and the final product might no longer meet the Dutch legal definition of compost.

Gap 4: Exploring the fertilizing potential of mineral-emended compost

A noteworthy research gap lies in the exploration of how the introduction of minerals to compost might alter its fertilization capabilities. The hypothesis posits that the inclusion of minerals could potentially enhance the efficient sequestration of organic matter, leading to a decelerated process of mineralization. This outcome could subsequently result in a reduced availability of nutrients, either in terms of diminished overall nutrient content or a delayed release rate. Obtaining this knowledge is crucial for making informed decisions on optimal composting practices and for effectively harnessing compost as a sustainable fertilization source.

Recommendation: By systematically examining the interplay between mineral amendments, organic matter sequestration, and nutrient availability, we can gain valuable insights into the potential trade-offs and benefits associated with mineral-amended compost utilization.

Gap 5: BNI potential in the agronomic and regulatory context of maize cultivation on sandy soils

Overall, most of the studies on BNI potential refer to lab experiments, of which a considerable part are not executed with soil as a growing medium. In the field, multiple above- and belowground processes could influence the performance of BNI crops and the distribution of BNI compounds in the soil matrix. Even more, the soil matrix itself will play a major role in nitrification processes. It is therewith difficult to predict from ex-situ experiments how BNI crops will influence nitrification processes and N_2O emissions in the field. This implies there is much room improving knowledge on the actual potential of BNI crops in the field.

Recommendations: It is highly recommended to not only focus on the fundamental BNI mechanisms, but also to study the potential of crops with BNI potential which could be incorporated in maize cropping on sandy soil in field experiments. With a sound set-up, the BNI potential of different cover crops with BNI potential in a maize/sorghum and grass-clover rotation system on sandy soil in time and space could be studied. It is important to take the year-round N_2O emissions into account, or to find another indicator to quantify net changes in soil N-cycling when integrating BNI crops in the farming system.

From practice we know that crop management differs per farmer depending on the previous grown crop, soil properties, weather conditions and time of year. Therefore, it is also recommended to consider the effect of 1) different fertilizer types and rates, 2) soil pre-treatments (e.g. tillage, cultivator, harrow, no till), 3) strategies for cover crop incorporation in early spring, and 4) a range of weather conditions on the BNI contribution to soil N-cycling.

Gap 6: Methods for assessing direct effects of BNI compounds in a field context

Priority should be given to assessing the effect of potential BNI crops on N-cycling in the field context. Next to that, the linkage between crop traits in the field and N-cycling is still a topic to be further studied. Finding suitable sampling and measuring methods will be part of the challenge. A combination of field and incubation experiments could be used to improve the mechanistic understanding of crop induced nitrification inhibition in the field.

Recommendations: Next to measuring N-cycling in the field as affected by BNI crops, PCR and microscopic techniques could be used to assess the effect of BNI crops on the microbial community composition. Directly measuring correlations between BNI compounds release and AO activity under field conditions is difficult to accomplish. Therefore, controlled environment rhizotron set-ups could be applied to find interrelations between N-cycling (raisin bags for NO_3^-), plant leachates (microcups, suction filters) potentially containing BNI compounds and AO communities (PCR, microscopy). It is recommended to grow BNI crops on field soils in these experiments to capture the field situation as much as possible. BNI compounds in root leachates could be analyzed through e.g. HPLC or LC-/GC-MS techniques. Collected compounds could also be used for incubation experiments, to study how these affect AO community activity.

It is recommended to analyze plant rhizodeposits during different phases of the BNI crop cycle to find out when they are peaking in release of BNI compounds. Also, it is recommended to measure the concentration of BNI compounds after different times of incorporation of plants in the soil. This again could be linked to dynamics of microbial populations.

To learn more about the interaction between nitrification and root architecture, it is suggested to measure root architecture and active root tips, rooting depth and width next to $N-NH_4^+$ uptake rates, $N-NO_3^-/N-NH_4^+$ ratio and aromaticity of root exudates (e.g. polyphenolics).

Gap 7: Shifting to a more NH_4^+ strategy: effect on SOM mineralization

The use of BNI crops is intended to counteract potential adverse effects of nitrous oxide emissions after compost addition. However, as BNI crops theoretically shift the soil to a more NH_4^+ dominated system, this in turn could enhance SOM mineralization (Subbarao et al., 2021). It is suggested to take this feedback loop into account and carefully assess the "climate smart" compost – BNI cropping system as a whole, including CO_2 and CH_4 emissions.

5   Conclusion

The novel strategies of incorporating clay minerals into composting and utilizing crops with biological nitrification inhibitors hold significant promises for respectively enhancing soil

carbon sequestration and mitigating nitrous oxide emissions. However, addressing the identified knowledge gaps is crucial to fully capitalize on these approaches. Understanding the different pathways of soil organic matter formation, the intricate interactions between soil mineralogy and organic matter, the fertilizer capabilities of mineral amended compost, the practical potential of BNI crops in practical conditions, suitable methods for assessing direct effects of BNI compounds in the field, and implications of increasing the soil NH_4^+ concentrations are essential for successful implementation. By closing these knowledge gaps, we may be able to develop effective climate-smart agricultural practices that contribute substantially to the goals of the Paris Climate Agreement and the European Union in combating climate change and ensuring sustainable soil management for a resilient and productive agricultural future.

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