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Exploring the Roles of Iron in Soil nitrogen processes

By

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DISSERTATION

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DOCTOR OF PHILOSOPHY

in

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of the

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DAVIS

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EXECUTIVE SUMMARY

Attempts at understanding controls and drivers of soil nitrification, the process by which ammonium (NH_4^+) is converted to nitrate (NO_3^-), often omit the role of iron (Fe) minerals. However, these minerals are widespread components of soils and sediments and their involvement in both the enzymatic and non-enzymatic reactions that influence the N cycle, including nitrification, is well-known. In soils and sediments experiencing fluctuation in pH or redox conditions, Fe often coprecipitates with organic carbon (OC), which yields iron-organic coprecipitates (Fe-OC) or flocs. These soil components are critical to stabilizing carbon (C) against microbial decay and determining Fe reactivity, which may limit Fe participation in nitrification. Moreover, Fe-OC flocs may affect nitrification by controlling the availability of trace metals (e.g., Fe, molybdenum (Mo), etc.) and nutrients that are required for microbial growth, metabolism, and activity. In this dissertation, I explored these possible interactions with the goal of providing mechanistic descriptions of how Fe-OC affect nitrification in agricultural soils. The dissertation document is structured around 3 chapters:

❖ In the first chapter, I have taken a general approach and reviewed the processes by which Fe affects N bioavailability in soils. To do so, I categorized these processes into four different categories/roles. In fact, Fe affects N bioavailability directly by acting as a sorbent, catalyst, and electron transfer agent, or indirectly by promoting certain soil features, such as aggregate formation and stability, which affect N turnover processes. Then, I explored the possible outcomes of these roles on N bioavailability as influenced by soil environmental conditions, such as redox status. Finally, I highlighted research needs for each category of roles and detailed the analytical framework needed for a complete understanding of Fe-N interactions in soils. This work is currently under review for the journal Biogeosciences. Slimani, I., Barker, X.-Z., Lazicki, P., and Horwath, W.: Reviews and syntheses: Iron: A driver of nitrogen

bioavailability in soils?, Biogeosciences Discuss. [preprint], https://doi.org/10.5194/bg-2022-194, in review, 2022.

❖ In the second chapter, I researched the mechanisms by which Fe-OC flocs affect nitrification in agricultural soils. To do so, I used flocs of different chemistry (aromatic and aliphatic) and known Fe and C composition to investigate their effects on nitrification in soils along a soil C gradient. I found that in mineral soils (< 3% C soil), Fe-OC flocs dramatically reduce net nitrification by restricting the availability of molybdenum (Mo) to the nitrifying communities. In fact, these microbes use Mo as cofactor to oxidize nitrite (NO_2^-) to NO_3^-. This reduction in Mo bioavailability possibly originates from its incorporation into or adsorption to flocs or their decomposition products. In contrast to mineral soils, Fe-OC flocs reduced net nitrification to a lesser extent in organic soils (>3% C), likely because organic matter limited floc adsorption capacity and/or released Mo through mineralization.

❖ In the third chapter, I was intrigued that, even though Fe-OC flocs decreased net nitrification by restricting Mo bioavailability, supplying Mo to a soil did not reverse their effects on nitrification. I found that beside affecting Mo bioavailability, Fe-OC flocs changed soil nutrient status. Generally, flocs increased water-soluble Fe, copper (Cu), nickel (Ni), phosphorus (P), zinc (Zn), manganese (Mn), magnesium (Mg), aluminium (Al), cobalt (Co), calcium (Ca) and potassium (K)) while decreasing water-soluble Mo in mineral soils. We interpreted these results in the light of the current knowledge on the influence of nutrient on microbial processes. We apply the principle of the Liebig's Law of the Minimum and knowledge on nutrient toxicity to microbial communities to build a conceptual framework of the possible links among Fe-OC flocs, soil nutrient status and nitrification in soils.

The results of this dissertation give new insight into mechanisms by which Fe affects nitrification in soils. Also, because flocs originate from engineering systems like wastewater treatment and their retention in wetlands was

proposed as a strategy to build soil C stock and reverse land subsidence in wetlands, it is important to address their effects on N cycling processes. Our results suggest that Fe-OC flocs can be used as nitrification inhibitors in mineral soils, which can alleviate other environmental issues such as eutrophication of lakes and water pollution. However, more research on their effects on other N processes, such as denitrification and N immobilization are needed.

CHAPTER I: Reviews and syntheses. Iron: A driver of nitrogen bioavailability in soils?

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Abstract. An adequate supply of bioavailable nitrogen (N) is critical to soil microbial communities and plants. Over the last decades, research efforts have rarely considered the importance of reactive iron (Fe) minerals in the processes that produce or consume bioavailable N in soils, compared to other factors such as soil texture, pH, and organic matter (OM). However, Fe is involved in both enzymatic and non-enzymatic reactions that influence the N cycle. More broadly, reactive Fe minerals restrict soil organic matter (SOM) cycling through sorption processes, but also promote SOM decomposition and denitrification in anoxic conditions. By synthesizing available research, we show that Fe plays diverse roles in N bioavailability. Fe affects N bioavailability directly by acting as a sorbent, catalyst, and electron transfer agent, or indirectly by promoting certain soil features, such as aggregate formation and stability, which affect N turnover processes. These roles can lead to different outcomes on N bioavailability, depending on environmental conditions such as soil redox shifts during wet-dry cycles. We provide examples of Fe-N interactions and discuss the possible underlying mechanisms, which can be abiotic or microbially meditated. We also discuss methodological constraints that hinder the development of mechanistic understanding of Fe in controlling N bioavailability and highlight the areas of needed research.

1 Introduction

Terrestrial ecosystem productivity is largely constrained by nitrogen (N) availability (Vitousek and Howarth, 1991). The largest pool of N in these ecosystems is found in soils which contains 133–140 Pg of total N globally within the first top 100 cm of soil (Batjes, 1996). A clear description of the factors controlling N bioavailability in soils is needed to design agricultural practices that meet crop demand and mitigate N loss to the environment. A large literature exists on the effects of soil texture, OM, mineral N inputs, pH, moisture, and microbial communities on N mineralization. However, geochemical factors, such as reactive Fe minerals, are rarely considered in N cycling, though they are often studied as vital components of carbon (C) cycling. Since C and N cycles are interconnected in soils (Feng et al., 2019; Gärdenäs et al., 2011), they should be regulated by the same factors, including mineralogy type (Wade et al., 2018). Moreover, a series of observations in the literature highlight the involvement of Fe in N dynamics:

(a)   A large proportion of SOM is contained in associations with Fe minerals (Lalonde et al., 2012; Wagai and Mayer, 2007). The close proximity between the two components can trigger a myriad of interactions, including OM stabilization.

(b)   Fe is a redox-active mineral that cycles between two redox states (Fe(II) reduced; Fe(III) oxidized). Fe(II)/Fe(III) redox transformations are tightly coupled with N cycling reactions (Kappler et al., 2021; Li et al., 2012a).

(c)   A myriad of interactions (Fig. 1) between Fe and N cycles have been observed in soils. These reactions, which can occur through both chemical or microbial pathways, include chemo-denitrification (Burger and Venterea, 2011) and anaerobic ammonia oxidation coupled with Fe(III) reduction- Feammox (Wan et al., 2021). In addition, Fe is shown to affect rates of denitrification (Wang et al., 2016) and nitrification (Huang et al., 2016a) in experiments with both Fe addition and soil endogenous Fe (Han et al., 2018).

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(d) Increasing evidence shows that Fe represents a major control over N processes. For example, Fe (III) minerals and Fe complexed with SOM explained nitrous oxide (N_2O) emissions across a set of agricultural soils; more than any other intrinsic soil property (Zhu et al., 2013). Similarly, Han et al. (2018) found that soil Fe regulates N_2O emissions. By using structural equation modeling, Wade et al. (2018) found that Fe oxides strongly mediate N mineralization in agricultural soils.

(e) Fe is involved in the enzymatic processes in the N cycle. For example, dissimilatory nitrate reductase, which catalyzes the first step in denitrification, contains Fe as a component of the internal electron transfer chain. Similarly, nitrite oxidoreductase, which catalyzes ammonia oxidation to nitrite, contains Fe-rich cytochromes. Fe also regulates the expression of proteolytic genes responsible for protease production (Maunsell et al., 2006).

Therefore, the impacts of Fe on N cycling can be significant and should be considered. This review aims to understand the roles of Fe in controlling N bioavailability. To do so, we categorize the processes by which Fe affects OM dynamics into four different categories/roles. In the **sorbent role**, OM interacts with Fe(III) through adsorption, coprecipitation or surface coatings (Eusterhues et al., 2005; Lalonde et al., 2012; Wagai and Mayer, 2007). These associations increase OM storage by decreasing its availability to extracellular enzymes and decomposition processes (Lalonde et al., 2012). In fact, the content of Fe minerals is a major predictor of soil sorptive capacity (Mayes et al., 2012). In the **structural role**, Fe minerals participate in the formation of soil aggregates (Zhang, X. et al., 2016) and increase soil structural stability (Barral et al., 1998; Xue et al., 2019). Aggregates can increase OM stability and retention in soils by protecting it from the decomposer community and their enzymes (Kleber et al., 2021; Van Veen and Kuikman, 1990). Moreover, Fe(III) can facilitate the formation of large polymers of OM that promote its stability. Thirdly, Fe's **electron transfer role** depends on its oxidation state. Fe(III) serves as a sink of electrons, while Fe(II) functions as a source of electrons. During anoxic periods, dissimilatory Fe(III) reduction can be coupled with the oxidation of OM, which accounts for significant amount

of C loss under anoxic conditions (Dubinsky et al., 2010; Roden and Wetzel, 1996). This process can release previously adsorbed or coprecipitated C, thereby increasing its susceptibility to degradation. Finally, Fe has a **catalysis role**, whereby Fe acts as a catalyst for the production of reactive oxygen species (ROS), which are potent oxidants of OM. This happens through Fenton reactions that are prevalent in various soils such as cultivated soils (Chen et al., 2020; Hall and Silver, 2013), arctic soils (Trusiak et al., 2018) and desert soils (Georgiou et al., 2015; Hall et al., 2012). These reactions are an overlooked but potentially important pathway for OM transformation in soils and sediments and N bioavailability (Kleber et al., 2021; Lipson et al., 2010; Merino et al., 2020; Trusiak et al., 2018; Wang et al., 2017).

While these roles of Fe in controlling C cycling have been studied extensively, their effects on N bioavailability are not well explored. This review seeks to underpin these suggested relationships and provide mechanistic descriptions of how Fe controls N bioavailability in soils. This information is needed to construct reliable models with improved predictive power of N cycling in terrestrial ecosystems (Wade et al., 2018), and will offer new possibilities for land management.
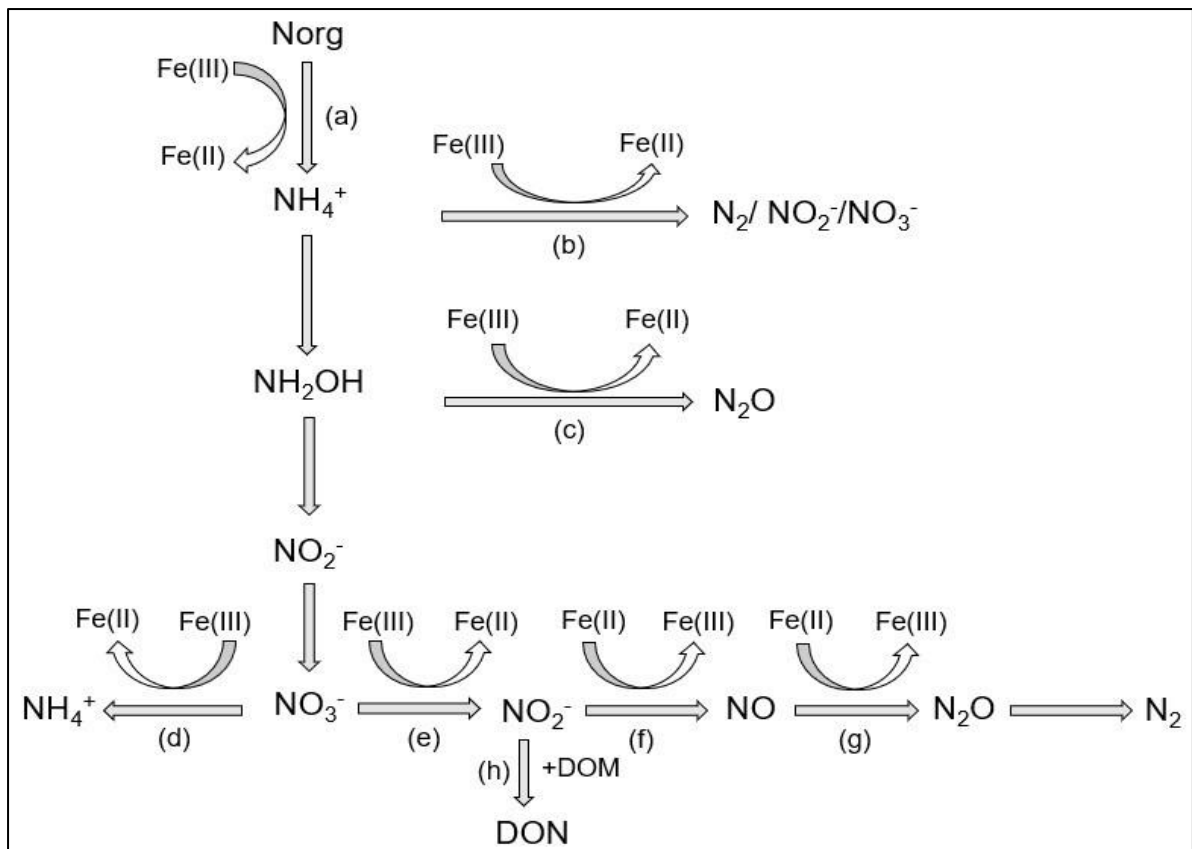
Figure 1. Fe interacts with N cycles at various steps: (a) mineralization, (b) feammox, (c) N$_2$O production by Fe-mediated hydroxylamine oxidation, (d) anaerobic reduction of nitrate to ammonium, (e)+(f)+(g) Denitrification, (h) Fe-meditated abiotic formation of dissolved organic nitrogen (DON) by reaction of nitrite (NO$_2^-$) with dissolved organic matter (DOM).

2 Fundamental concepts to understand Fe-N interactions

The interactions of Fe and N depend on numerous characteristic properties of Fe and N compounds, which are briefly described below. The soil environment has the capacity to shape these interactions through changing these properties.

2.1 Iron

Iron oxides, hydroxides and oxyhydroxides (collectively referred to as iron oxides (Fe-oxides)); are widespread redox-active minerals in soil and sediments. Although Fe is relatively abundant in soils, the amount of its bioavailable fraction is low (Colombo et al., 2013). In oxic soils, Fe mainly exists as poorly soluble Fe(III) oxides. In poorly drained soils experiencing periodic or transient anoxia, Fe(III) oxides undergo reductive dissolution, through both chemical and biotic pathways, which releases soluble Fe(II). This process is controlled by pH and redox potential (Eh) of soil, which control the distribution between Fe(III) and Fe(II) species.

Fe(III) oxides exist in a variety of polymorphs with unique physical and chemical properties controlling their reactivity (Navrotsky et al., 2008). These minerals have variable surface charge generated by the protonation-deprotonation of surface hydroxyl groups, which provide a significant proportion of pH-dependent negative charge in soils (Sumner, 1963). The presence of pH-dependent charged groups influences the interactions with OM. When their relative point of zero charge (PZC) is below soil pH, Fe oxides exhibit positively charged surfaces and sorb negatively charged OM; however, if PZC is above soil pH, OM will be repulsed from the negatively charged Fe minerals.

The reactivity of an Fe mineral is also driven by surface topography, particle size and crystallinity. First, surface topography defects, which arise from deviations in the ideal composition and/or structure of minerals, play an important role in a variety of surface processes. Defect sizes and content are closely related to the strength and adsorption capacities of minerals (Li et al., 2015a), as they offer potential binding sites for organic molecules and promote the formation of stronger surface complexes (Petridis et al., 2014). Moreover, Fe(III) minerals with few defects are less vulnerable to reductive dissolution in favor of the ones with more defects (Notini et al., 2019). Second, small particles possess high specific surface area (SSA), which enables high adsorption capacity. Finally,

less crystalline and more disordered phases react readily because they typically have a much larger and more reactive SSA as well as higher solubility compare to crystalline phases (Schwertmann, 1991).

Additionally, Fe oxide reactivity is altered by the presence of OM coatings on mineral surfaces (Gao et al., 2018; Kleber et al., 2007; Poggenburg et al., 2018) and the type of coverage (monolayer vs. multilayer coverage). For instance, adsorbed organics can inhibit the development of crystals (Boland et al., 2014; Henneberry et al., 2016), halt the reductive dissolution by surface passivation, reduce the amount of binding sites available for sorption (Kaiser and Zech, 2000a), or limit Fe(II) oxidation under oxic conditions (Daugherty et al., 2017).

2.2 Nitrogen

N in soils is made available to plants and microbes by N mineralization. i.e., the process by which organic N (ON) is decomposed to mineral forms of N (MinN: ammonium (NH_4^+) and nitrate (NO_3^-). ON predominates over MinN forms and can make up to 95 % of the bulk soil N in some surface soils (Knicker, 2011; Schulten and Schnitzer, 1997). ON exists in various chemical forms (Box 1), with a predominance of proteins and peptides. N in these compounds is generally not directly bioavailable due to molecular size constraints on microbial cell uptake (Schimel and Bennett, 2004). Depolymerization reactions, carried out by the activity of extracellular enzymes (EE), transform these polymers into soluble, low molecular weight (MW) organic monomers (e.g., short oligopeptides, amino acids). These reactions have long been considered the rate-limiting step in soil N cycling (Schimel and Bennett., 2004), however, recent research suggests that substrate availability can be as or more important (Noll et al., 2019). As both peptidase activity and protein sorption are affected by Fe minerals, Fe may drive gross amino acid (AA) production in soils. Once mineralized, N monomers are subject to three possible fates: first, they can be directly utilized by soil microorganisms or plants (Farrell et al., 2011; Geisseler et al., 2010). Second, they can be transferred into associations with soil minerals (mineral-associated organic nitrogen; MAON) and further occluded within soil aggregates. Finally, they can be further mineralized to NH_4^+, due to the

activities of extracellular and intracellular enzymes such as urease and AA oxidases (Geisseler et al., 2010). Recent research shows that the size of AAs available for mineralization is controlled by peptidase activity, but more so by substrate (protein) availability, both of which are affected by the interactions with Fe minerals. Therefore, Fe may drive gross AA production in soils (Noll et al., 2019).

Box 1: Chemical forms of organic N in soils

Soil ON exists predominantly as protein and peptides, and to a lesser extent as amino-sugars and nucleic acids (Kögel-Knabner, 2006). Proteins are intrinsically reactive towards soil minerals, due to a number of properties, including hydrophobicity, surface charge distribution, surface area, number and type of functional groups, conformation, and size. For instance, smaller proteins often have fewer available sites for sorption (Lützow et al., 2006). Protein decomposition is often equated with complete depolymerization to AAs, however, small peptides and AAs can be produced in equal amounts (Warren and Taranto, 2010). Relative to AAs, peptides are preferentially and rapidly utilized by microbes as sources of C and N (Geisseler et al., 2010; Farrell et al., 2011; Farrell et al., 2013; Hill et al., 2012) . Amino-sugars, which account for 5–8% of ON (Amelung et al., 1996), comprise chitin and peptidoglycan (PGN) and other components of microbial cell walls. The important contribution of amino- sugars to bioavailable N in soils is debated (Martin and Haider, 1979; Kögel-Knabner, 2002; Roberts et al., 2007; Strickland and Rousk, 2010; Roberts and Jones, 2012; Hu et al., 2018). Finally, nucleic acids are generally decomposed by nucleases and yield individual nucleotides in soils. The chemical composition of these compounds may affect their decomposition dynamics. For instance, adenosine monophosphate is degraded faster than cytidine monophosphate (Therkildsen et al., 1996).

3 Sorbent role of Fe in controlling N bioavailability

3.1 Does extracellular enzymes sorption to Fe oxides affect their participation in N mineralization?

Soil microbes produce a variety of extracellular enzymes (EE) to acquire N, and increased N-acquiring enzyme activities correlate positively with N mineralization. These enzymes can be substrate-specific (e.g., proteases and aminopeptidases), or non-specific oxidative enzymes (e.g., laccase and peroxidase) (Caldwell, 2005; Sinsabaugh et al., 2009; Hassan et al., 2013), which are generally associated with C cycle, though their importance for N mineralization has also been demonstrated (Kieloaho et al., 2016; Zhu et al., 2014). Many of these enzymes become adsorbed to Fe minerals when released in soil. Such immobilization often lowers enzyme activities, increases their resilience to proteolysis (Sarkar and Burns, 1984; Rani et al., 2000; Tietjen and Wetzel, 2003; Kelleher et al., 2004), and allows for greater residence time in soils and more persistent activity (Yan et al., 2010; Schimel et al., 2017). However, opposing outcomes on enzyme activity have been reported (Quiquampoix and Ratcliffe, 1992; Quiquampoix et al., 1995; Servagent-Noinville et al., 2000). For instance, Fe adsorption reduced the activity of urease (Gianfreda et al., 1995; Bayan and Eivazi, 1999; Li et al., 2020), but increased the activity of N-acetyl-glucosaminidase (NAG) (Allison, 2006; Olagoke et al., 2020). These contradicting effects can have multiple explanations. First, enzyme active sites can become occluded, which limits the diffusion of N substrates towards the binding sites and lowers N decomposition as a consequence. Site occlusion is due to either conformational changes in the enzyme structure (Datta et al., 2017), Fe-induced aggregation (Olagoke et al., 2020) or unfavorable attachment orientation on mineral surfaces (Baron et al., 1999; Yang et al., 2019). Second, Fe oxides can inhibit the activity of EE by constraining N substrate availability. Along a 120-kyr-old chronosequence, Turner et al., (2014) found that Fe oxides inhibited the activities of urease and proteases more strongly than aminopeptidases, possibly due to the preferential adsorption of urea and proteins over peptides (Turner et al., 2014). Third, enzyme activity is likely affected by soil mineral content. Olagoke et al., (2020) observed that soil

with low mineral content offers a limited availability of adsorption sites, allowing less and weak bonding of enzymes with minerals with minimal impact on enzyme active site. Therefore, enzymes in mineral-poor soils may have high and more persistent activities than those in mineral-rich soils. In this case, the presence of functional and active EEs may allow microbes to invest in biomass production instead of enzyme production, which results in improved microbial C and N use efficiencies in mineral-poor soils, as hypothesized by (Olagoke et al., 2020). Other soil properties such as pH control enzyme sorption by affecting surface affinity and related binding strength and enzyme conformation (Quiquampoix et al., 1993). Finally, a new mechanism has been proposed recently by Chacon et al., (2019), who observed (experimentally) that goethite can induce the abiotic fragmentation of proteins and subsequent loss of activity (Chacon et al., 2019). The occurrence of this mechanism in soil and implications for enzyme activity and N bioavailability awaits further investigation and validation. Beyond adsorption, enzyme activity is affected by soil redox conditions. For instance, waterlogging treatments decreased the activity of urease (Pulford and Tabatabai, 1988; Gu et al., 2019) , whereas the activity of amidase was not affected (Pulford and Tabatabai, 1988). These effects were attributed to the production of reduced metals under waterlogged conditions, which may serve as inhibitors or activators of enzymes (Pulford and Tabatabai, 1988). Specifically, $Fe(II)$ was shown to stimulate the activities of oxidative enzymes under anaerobic conditions (Van Bodegom et al., 2005; Sinsabaugh, 2010), but strongly inhibit the activity of urease (Gotoh and Patrick Jr, 1974; Tabatabai, 1977). To conclude, Fe affects N-acquiring enzymes differently depending on the modalities of their interaction, enzyme and substrate identity, and soil properties and conditions. The direction and the magnitude of this effect may create distinct patterns of N bioavailability and enzyme activities across soils (Turner et al., 2014).

3.2 Does the sorption of N substrates to Fe oxides affect their bioavailability?

Many studies have demonstrated that poorly crystalline Fe minerals, such as ferrihydrite, control the sorption of N compounds in soils (Kaiser and Zech, 2000b; Dümig et al., 2012; Keiluweit et al., 2012a; Dippold et al., 2014).

Indeed, Fe minerals interact with a wide range of N-containing moieties via adsorption or coprecipitation processes; the latter process incorporates N into organo-mineral associations (MAOM), which are essential for OM stabilization (Leinweber and Schulten, 2000; Keiluweit et al., 2012b; Swenson et al., 2015; Heckman et al., 2018; Zhao et al., 2020). During these processes, Fe can form strong chemical bonds with N-containing moieties; for instance, goethite forms stronger bond with ammonia (NH_3) than with carboxylate, phosphate, or methyl groups (Newcomb et al., 2017). The bond strength between N and mineral surfaces varies considerably across different environments due to differences in the nature of binding mechanisms, mineral and N properties, soil properties such as pH and ion strength, and the presence of antecedent SOM on mineral surfaces (Lützow et al., 2006). However, protein may adsorb irreversibly to mineral surfaces over a wide range of solution pH and resist desorption (Hlady and Buijs, 1996; Yu et al., 2013); the latter mechanism is perceived to be a necessary step for EE to proceed with N mineralization. Similarly, nucleic acid molecules persist for a long time on clay minerals (Yu et al., 2013) and are shielded from degradation.

Advances in spectroscopic techniques have generated new conceptual models of organo-mineral associations, such as "the zonal structure model of organo-mineral associations", which postulates that organic compounds self-organize on mineral particle surfaces (Kleber et al., 2007). In this model, amphiphilic SOM compounds with N-bearing and oxidized functional groups directly interact with mineral surfaces to form "the contact zone", whereas hydrophobic groups face outwards creating a region of high hydrophobicity, "the hydrophobic zone". Additional organic molecules attach to this zone, forming an outer layer termed "the kinetic zone". Multiple recent observations support this model, including (1) the preferential enrichment of N-containing moieties on Fe mineral surfaces (Kopittke et al., 2018; Possinger et al., 2020), (2) the preferential adsorption of N compounds over other organic compound classes on Fe mineral surfaces (Gao et al., 2017) and (3) the partial sorption of some organic compounds, including AAs, to Fe minerals (Amelung et al., 2002; Dippold et al., 2014). This model has implications for N bioavailability, because, in contrast to the contact zone, the weakly sorbed N in the kinetic zone

likely exchange with soil solution and is more available. Recent research on the chemical composition of C and N at the organo-organic and organo-mineral interfaces of the model found that alkyl C and less N occurred at the former, whereas oxidized C and more N occurred at the latter (Possinger et al., 2020). The authors of this study hypothesized that the processes stabilizing C and N at these interfaces are different, considering that the association between SOM rich in O/N-alkyl C and Fe oxides explained the stabilization of O/N-alkyl C in soils (Schöning et al., 2005). In addition to protecting a fraction of bioavailable N, Vogel et al., (2014, 2015) found that sorption can retard the movement of N in soils, thereby increasing N retention by decreasing its accessibility to degradation mechanisms (Vogel et al., 2014; Vogel et al., 2015). More insight is needed to advance the understanding of N bioavailability from organo-mineral associations.

3.3 N sorption is counteracted by several destabilization mechanisms

The release of N from Fe-organic associations, or desorption, occurs due to several destabilization mechanisms, including surface displacement by competitive sorption, oxidative and reductive dissolution of Fe minerals (Kleber et al., 2015) and local disequilibrium in soil chemistry. Once released, SON may become accessible and vulnerable to microbial degradation or diffusion into microbial cells. The following is a discussion of the different destabilization mechanisms of Fe-organic associations in soils and factors influencing them:

(a) N desorption by oxidation and reductive dissolution of Fe minerals

The dissolution of Fe minerals, as a result of changes in soil pH or redox conditions, decreases their sorption capacity and compromises the stability of sorption complexes. When mineral dissolution occurs, Fe and OM enter the soil solution. For instance, chemical reduction of Fe(III) by sodium dithionate was shown to release C and N substances compared with no reduction (Bird et al., 2002). However, short-range order (SRO) Fe oxides can resist both chemical and microbial reduction, due to coprecipitation with aluminosilicates or physical protection within microaggregates (Henneberry et al., 2012; Shimizu et al., 2013; Eusterhues et al., 2014; Filimonova et al., 2016;

Suda and Makino, 2016; Coward et al., 2018; Tamrat et al., 2019). The extent of OM mobilized from mineral reduction remains unpredictable due to knowledge gaps related to their resistance mechanisms and their controls in soils. The oxidation of Fe(II) can also release OM by solubilizing Fe-organo associations via decreasing pH or by generating hydroxyl radicals through Fenton chemistry, which oxidize OM abiotically. Redox fluctuations can also affect OM cycling by changing mineral properties; for instance, such fluctuations can induce the transformation of amorphous Fe minerals into more crystalline forms, which can decrease OM stability and increase its turnover rates. However, mineral crystallinity was found to be positively correlated with SOM turnover rates (Hall et al., 2018) and was not associated with C release from Fe associations (Chen et al., 2020). These observations can be explained by the zonal structure of organo-Fe associations, in which OM in the kinetic zone can be lost, and the contact zone organics remain protected.

(b) N desorption by local disequilibrium in soil chemistry

OM in soils can be desorbed from mineral surfaces due to the establishment of local disequilibrium conditions. Such conditions result from depletion of DOM in the soil solution, due to microbial uptake, for example, promote the release of OM from MAOM until DOM concentrations in the soil solution are in equilibrium with sorbed OM. This process is likely affected by the strength of bonds between N substrates and Fe minerals; in fact, interaction forces vary considerably: strong interactions are favored by polyvalent cation bridges and ligand exchange whereas weak interactions occur by hydrogen bonds or van der Waals (Kleber et al., 2015). While the relationship between particular binding mechanism and N desorption from minerals has not yet been established in real soil conditions, multiple studies in model systems demonstrated that OM bound by ligand exchange was more resistant to desorption than other mechanisms (Wang and Lee, 1993; Gu et al., 1994; Gu et al., 1995; Mikutta et al., 2007). Therefore, it will be likely less affected by the dynamic equilibria principle and less N will be made available (Kleber et al., 2015).

(c) N desorption by surface displacement via competitive sorption

N associated with Fe can be displaced by the input of highly sorptive organic compounds. For instance, Scott and Rothstein (2014) observed that weakly bound, N-rich hydrophilic compounds were easily displaced by stronger binding compounds (e.g. hydrophobic compounds), leading to the downward migration of N to subsurface and mineral horizons.

(d) Is desorption of N from organo-mineral associations a prerequisite to N mineralization?

As mentioned earlier, desorption of protein from mineral surfaces is often perceived to be the primary pathway by which N substrates become accessible to microbial degradation (Schimel and Bennett, 2004). However, protein adsorption to Fe minerals is an irreversible process (Rabe et al., 2011), which restricts proteolytic activity. Recently, the direct proteolysis of protein at the mineral surface was investigated, as ferrihydrite- and goethite-adsorbed protein was found to be degraded without prior desorption (Tian et al., 2020). Substrate-enzyme complexes were formed directly at the surface of minerals. Together with the zonal structure of organo-mineral associations, this finding challenges the long-standing assumption that Fe minerals impair protein bioavailability through acting as a sorbent. The reader is referred to Keiluweit and Kuyper (2020) for a more expanded discussion of this mechanism (Keiluweit and Kuyper, 2020).

4 Structural role of Fe in controlling N bioavailability

4.1 Does structural Fe in clay minerals affect N bioavailability?

The majority of clay minerals contain Fe and account for 30-50 % of total Fe in soils and sediments (Favre et al., 2006; Stucki, 2013). Fe can be located in both the octahedral and tetrahedral sheets of 1:1 and 2:1 clay mineral or exist as coating on their surfaces (Stucki, 2013). N bioavailability can be affected by the redox cycling of this

structural Fe in clays. For instance, the reduction of structural Fe(III) allows the abiotic fixation of NH_4^+ (Zhang and Scherer, 2000; Deroo et al., 2021) through increasing negative charge and cation exchange capacity of clays (Pentráková et al., 2013). Further, the reductive dissolution of coated Fe on clay minerals promotes NH_4^+ diffusion into or out of clay interlayers (Zhang and Scherer, 2000). After de-fixation, the fixed NH_4^+ pool can serve as a source of bioavailable N (Deroo et al., 2021). In contrast to Fe(III) reduction, structural Fe(II) oxidation has not received much attention despite its possible involvement in processes that cause the loss of bioavailable N. For instance, Zhao et al., (2013) found that the oxidation of structural Fe(II) in nontronite causes the loss of NO_3^- as dinitrogen (N_2) (Zhao et al., 2013). The potential importance of such processes in N bioavailability should be considered, especially in highly weathered soils with high clay content.

4.2 Fe, soil aggregates and N bioavailability

Few studies have explored relationships between Fe, soil aggregates, and turnover of N in soils, despite multiple indications of their interconnection. First, Fe oxides are one of the most important constituents of soil microaggregates (Peng et al., 2015), serving as nuclei for their formation and meditating their stability (Barral et al., 1998; Pronk et al., 2012; Wei et al., 2016), acting as a cementing agent (Colombo and Torrent, 1991; Krause et al., 2020) and binding OM (Giovannini and Sequi, 1976; Totsche et al., 2017). Second, Fe oxides preferentially adsorb N-containing moieties. The observations that C:N ratio of sorbed organics decrease with decreasing particle size (Aufdenkampe et al., 2001) and increasing particle density (Sollins et al., 2006), suggest that N is an important component of microaggregate-SOM (Golchin et al., 1994). Indeed, using density fractionation, Wagai et al., (2020) observed joint accumulation of OM with low C:N ratio and pedogenic Fe and Al oxides in the meso-density fractions (1.8–2.4 g cm^{-3}) of five soil orders collected from different climate zones. Moreover, Rodionov et al., (2001) observed high concentrations of amino sugars in microaggregates (Rodionov et al., 2001). These observations have implications for N bioavailability, given the facts that mineral-associated OM compounds

located in stable aggregates has lower availability to microbes than those located on more accessible surfaces. Microaggregate-N is relatively more persistent than macroaggregate-N because microaggregates' turnover is relatively slow, which provides longer-term stabilization of OM (Cambardella and Elliott, 1993; Six et al., 2002). Krause et al., (2020) demonstrated that colloidal sized Fe promotes the formation of smaller-sized microaggregates (<20 µm). In addition, readily mineralizable N levels correlate positively with increased aggregate size in soils (Mendes et al., 1999), suggesting that Fe mediated micro-aggregation may slow down or suppress N mineralization. We hypothesize that there is another pathway by which Fe-promoted aggregation may decrease N mineralization. Aggregates of different sizes influence microbial community composition differently and therefore the activities of N mineralization enzymes (Muruganandam et al., 2009). Therefore, it will be useful to examine the distribution and the activities of these enzymes among soil aggregate size classes along a gradient of increased Fe mineral content in soils.

The relative importance of Fe in aggregate stability depends on several properties, such as Fe mineral and SOM content, mineral identity and degree of crystallinity, and soil redox conditions, which are expected to affect N bioavailability. In particular, Fe promotes the formation and stability of aggregates in soils with low OM and high Fe content (Barral et al., 1998; Wu et al., 2016). Duiker et al., (2003) showed that poorly crystalline Fe minerals are more important than crystalline minerals for aggregate stabilization (Duiker et al., 2003). Partial or complete removal of mineral-forming components, for example due to Fe reduction, can initiate aggregate turnover and destabilization (Michalet, 1993; Cornell and Schwertmann, 2003) which will eventually expose associated OM to microbial degradation (Lützow et al., 2006). Indeed, Cambardella and Elliott (1993) found that the loss of aggregates caused organic carbon (OC) and ON loss from SOM (Cambardella and Elliott, 1993). Silva et al., (2015) reported that applying Fe-rich biosolids in a tropical soil chronosequence induced rapid formation of microaggregates and significantly increased SOC (Silva et al., 2015). Similarly, Bugeja and Castellano (2018)

observed positive correlation between ammonium oxalate-extractable Fe (AmOx-F), C and N in microaggregate, indicating that Fe and microaggregate stabilization are interconnected (Bugeja and Castellano, 2018).
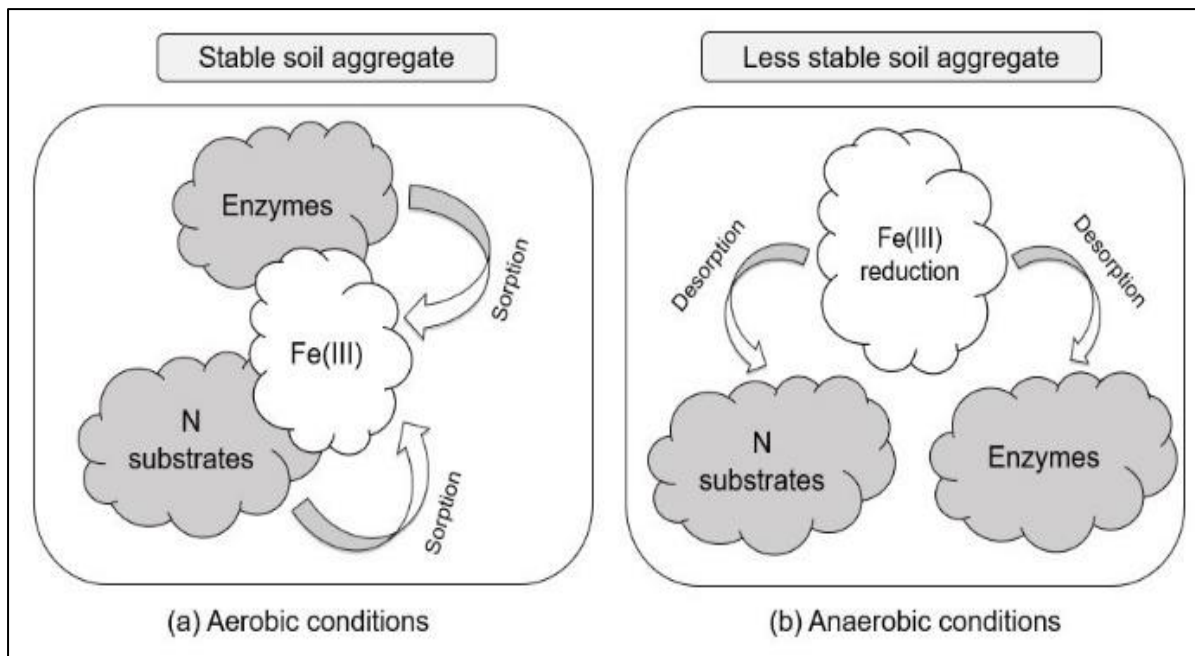
Figure 2. (a) Fe minerals sorb both enzymes and N substrates and promote stability of microaggregates, which offers protection to N from degradation in soil under oxic conditions. (b) Fe reduction releases N substrates and may lead to aggregate destabilization in soil under anoxic conditions.

4.3 Does Fe-induced ON polymerization increase the recalcitrance of N?

Little is known about Fe (mineral)-induced OM polymerization in soils. Some evidence exist that Fe oxides induce both C and N polymerization of SOM (Piccolo et al., 2011; Li, C. et al., 2012; Johnson et al., 2015; Zou et al., 2020) . In a long-term organic fertilization experiment, Yu et al. (2020) proposed that the Fe-catalyzed formation of reactive oxygen species (ROS) allows C monomers to recombine into large, recalcitrant C biopolymers through the formation of intramolecular bonds. A similar process was observed by Piccolo et al. (2011). Similarly, hydrohematite, maghemite, lepidocrocite and hematite can induce the oxidative polymerization of hydroquinone, with rates depending on the type of minerals (Huang, 1990). Synthetic ferrihydrite and goethite were demonstrated

to induce peptide bond formation between aspartate chains (Matrajt and Blanot, 2004), as well as the abiotic formation of AAs from simple organics such as pyruvate and glyoxylate (Barge et al., 2019). The environmental conditions in these experiments were similar to those occurring in natural systems such as in Fe-containing sediments (Barge et al., 2019). More studies of abiotic polymerization by minerals must be envisaged given that sorption is a ubiquitous and naturally occurring phenomenon in soils.

5 Catalytic role of Fe in controlling N bioavailability

Emerging research has revealed that ROS derived from Fe-catalyzed Fenton reactions (Box 2) are implicated in N mineralization. These reactions may involve abiotic or coupled biotic-abiotic processes causing N to mineralize, as explained below. In desert soils, the reaction of light with hematite generates ROS, which can oxidize AAs to nitrous oxide (N_2O) (Georgiou et al., 2015) and N oxide gases (Hall et al., 2012). Compared to soil containing water, desert soils accumulate photogenerated superoxides and peroxidases via complexation of $O_2^{\cdot-}$ with surface transition metal oxides. When these soils are wetted, the accumulated ROS are subjected to dismutation and hydrolysis leading to the generation of $HO^\bullet$ and subsequent OM oxidation. While this mechanism is strictly abiotic, soil microorganisms in diverse ecosystems were found to use Fe-generated $HO^\bullet$ to acquire organic C and N (Diaz et al., 2013; Shah et al., 2016; Zhang, J. et al., 2016; Op De Beeck et al., 2018). For instance, a boreal forest fungus (*Paxillus involutus*) may use radical oxidation to stimulate N mineralization in various ways (Op De Beeck et al., 2018): (1) to liberate NH_4^+ from amine groups of proteins, peptides, and amino acids according to mechanisms reviewed in Stadtman and Levine (2003), (2) to facilitate the accessibility of protein-N in SOM complexes to proteolytic degradation and (3) to enhance protein vulnerability to proteolysis and increase the activity of proteolytic enzymes (Zhang, J. et al., 2016).

Despite their involvement in N liberation, ROS may promote the formation of stable and protective Fe-associated OM complexes. In a long-term fertilization experiment conducted by Yu et al., (2020), Fe mobilized by Fenton reactions formed new short-range order (SRO) Fe minerals, which promoted C and N storage. Moreover, ROS generated from catalytic reactions involving Fe can also cause enzyme oxidation and subsequent loss of activity (Huang et al., 2013).

Box 2: Fe-catalyzed Fenton reactions

Most Fe minerals, such as ferrihydrite, goethite, hematite, magnetite, and pyrite, can catalyze Fenton-like reactions (Kwan and Voelker, 2003; Garrido-Ramírez et al., 2010). Fe-catalyzed Fenton reactions are mainly driven by fluctuating redox conditions (Xu , J.et al., 2013), oxygenation of Fe^{II}- bearing minerals (Tong et al., 2016) and photochemistry (Georgiou et al., 2015). Despite having a short lifetime in soil (Apel and Hirt, 2004), ROS, such as $HO^{\bullet}$ (E^o = 2.8 V), are non-selective and strong oxidants of OM (Gligorovski et al., 2015).

Photoreduction of Fe(III)-ligand (L) complexes : Fe(III)-L + hv -> Fe(II) + L*

Reactions of Fe meditated ROS generation:

$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + HO^{\bullet}$

$Fe(II) + H^+ + HO_2^{\bullet} \rightarrow Fe(III) + H_2O_2$

$Fe(II) + O_2 + H^+ \rightarrow Fe(III) + HO_2^{\bullet}$
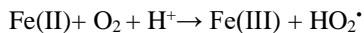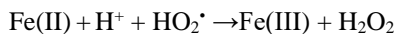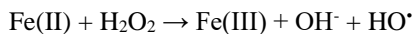
6 Electron transfer role of Fe in N bioavailability

Electron transfer to Fe(III) oxides, both biotically or abiotically, is a critical step in many processes favoring the gain or the loss of N from soils and sediments (Ding et al., 2014; Sahrawat, 2004). The ability of Fe(III) minerals

to accept electrons, or their 'reducibility', varies greatly with crystallinity, particle size, solution pH, ambient Fe(II) concentration, the presence of adsorbates and aggregation level (Roden, 2004; Roden, 2006). Here, we explore relationships between mineral reducibility and anaerobic NH_4^+ oxidation associated with Fe reduction (Feammox) and anaerobic OM oxidation to illustrate two examples of N processes that are involved in bioavailable N production and loss. Starting with Feammox, this process occurs mostly in acidic soils and has been estimated to metabolize 7.8−61 kg NH_4^+ /ha/year in paddy soils, accounting for about 3.9 %−31 % of N fertilizer loss (Ding et al., 2014). The terminal products of this process are either N_2, NO_2^- or NO_3^- with N_2 as the dominant product (Yang et al., 2012). Feammox rates are strongly positively correlated with the concentrations of microbially reducible Fe(III) (Ding et al., 2014; Li et al., 2015b; Ding et al., 2019; Ding et al., 2020). Moreover, Fe(III) enhances the activity, distribution and diversity of microbial communities involved in Feammox (Huang, S. et al., 2016; Ding et al., 2017). A series of incubation studies investigated the effects of different Fe sources on Feammox, and the results demonstrated that only ferrihydrite and goethite, not ferric chloride, lepidocrocite, hematite, or magnetite, served as electron acceptors for Feammox (Huang and Jaffé, 2015; Huang and Jaffé, 2018). These observations can be explained by a possible accumulation of free Fe(II), which halted Feammox, or due to the limited ability of Fe-reducers in reducing certain minerals (Huang et al., 2014). It is notable that chelates (Park et al., 2009) and electron shuttles (Zhou et al., 2016) can facilitate electron transfer to Fe(III) minerals (Fig. 3), which enhances their reduction rates and related N processes. For instance, the addition of electron shuttles increased potential N loss by Feammox by 17–340% compared to no addition (Zhou et al., 2016). Similar to Feammox, NH_4^+ production rates in submerged soils and sediments were found to be strongly correlated with reducible Fe(II) production rates (Sahrawat and Narteh, 2001; Sahrawat, 2004).
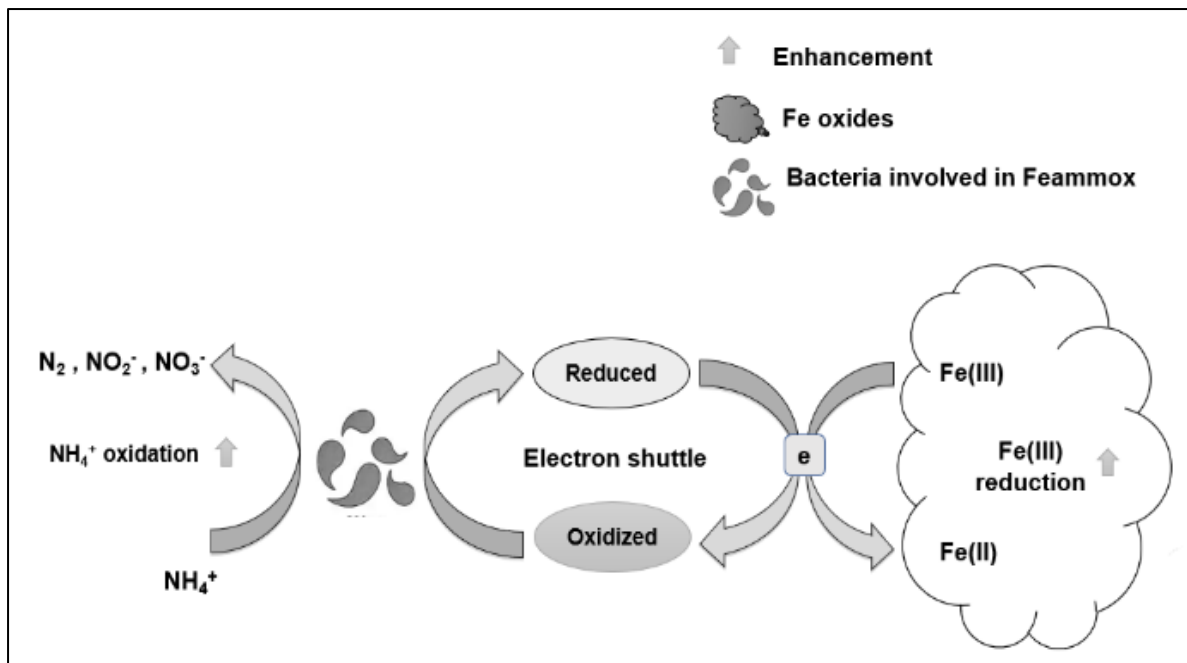
Figure 3. Electron shuttles enhance Fe reduction and NH4+ oxidation (Feammox) rates.

The electron-donating capacity of Fe minerals is also involved in N bioavailability. In fact, many $Fe(II)$ species, including soluble $Fe(II)$- and $Fe(III)^-$bearing minerals such as siderite and magnetite, can act as electron donors (Benz et al., 1998; Chaudhuri et al., 2001) for NO_3^- reduction coupled with Fe oxidation, which promotes the loss of NO_3^- as gases. For denitrification, it was found that N_2O emissions from flooded soils with contrasting $Fe(II)$ levels were regulated by $Fe(II)$ electron donating capacity: the electrons donated reached 16.2% and 32.9% in soils with low and high $Fe(II)$ content, respectively. Soil with high $Fe(II)$ content emitted less N_2O and more N_2, suggesting an improved denitrification efficiency due to an electron flow which exceeded the demand for N_2O production (Wang et al., 2016).

7 Involvement of Fe in soil phenomena that affect N bioavailability

7.1 Priming

Priming occurs when new input of labile C influences (positive or negative) the decomposition of native SOM (Kuzyakov et al., 2000). Several mechanisms have been proposed to explain this effect, including a shift in microbial communities (Fontaine et al., 2003), microbial N mining (Craine et al., 2007) and microbial activation (Drake et al., 2013). However, investigations of the patterns and drivers of priming across both local and broad geographical scales indicate that SOM stabilization mechanisms, including associations with Fe oxides, regulate priming and explain most of its variation (Chen et al., 2019; Jeewani et al., 2021a). In fact, positive priming, which occurs when new inputs increase SOM mineralization, is negatively related to MAOM concentration due to Fe constraining the accessibility of sorbed organics to microbial degradation (Bruun et al., 2010; Porras et al., 2018). Thus, the disruption of Fe-organic associations can lead to positive priming by liberating sorbed C and N compounds and making them more accessible. In the rhizosphere, this process takes place chemically when plant exudates strip Fe from Fe-organic associations by surface complexation, displacement of sorbed organics into soil solution (Keiluweit et al., 2015) and reductive dissolution of Fe (Zinder et al., 1986; Ding et al., 2021). Biotically, root exudates can activate microbes by providing C and energy, leading to increased production of N-acquiring enzymes and subsequent N mineralization (Yuan et al., 2018; Jilling et al., 2018; Jiang et al., 2021; Jilling et al., 2021).

The magnitude of priming depends on the extent to which these aforementioned destabilization pathways affect Fe-organic associations. For instance, Li, H. et al., (2021) showed that MAOM on ferrihydrite is susceptible to both abiotic and biotic pathways, whereas MAOM on goethite is more susceptible to abiotic pathways (Li, H. et al., 2021). Therefore, the ability of microbes and plant communities to secrete specific exudates capable of

triggering specific destabilization pathways of the dominant mineral in their environment will affect how much N can be made available from mineral associations (Jilling et al., 2018; Li, H. et al., 2021).

7.1.1 Fe-mediated priming in soils under reducing conditions

Recently, Fe-mediated priming in soils under reducing conditions has received growing interest. Dunham-Cheatham (2020) found that glucose application to a soil under anoxic-oxic transition induced a novel type of priming by facilitating the reductive dissolution of Fe^{III}-C associations under anoxic conditions followed by a dramatic increase of OC mineralization when oxic conditions were restored (Dunham-Cheatham et al., 2020). Li, H. et al., (2021) found that the roles of Fe in anaerobic OM mineralization can be shifted by microbial biomass C (MBC). In soil with low MBC, both ferrihydrite and goethite protected the added acetate from decomposition through sorption processes. In soil with high MBC, however, goethite acted as an electron acceptor and increased acetate decomposition, whereas ferrihydrite predominantly adsorbed the added substrate. Priming decreased in both low and high MBC soils, but more in low MBC soil (Li, H. et al., 2021). Lecomte et al., (2018) demonstrated that Fe(III)-reducing microorganisms have a competitive advantage of colonizing plant roots in the rhizosphere due to their capacity of providing Fe(II) for plant nutrition in exchange for C-rich exudates and performing denitrification (Lecomte et al., 2018). These exudates are probably used as a C source in the denitrification process or to destabilize Fe-organic associations and release sorbed C and N (Dunham-Cheatham et al., 2020). More research into Fe-mediated priming in strictly anoxic soils, or at the oxic-anoxic transition, is needed.

7.1.2 Fe may affect priming by shaping microbial community composition

Fe oxides may alter microbial community composition and soil C and N content (Heckman et al., 2009; Heckman et al., 2018), likely through controlling nutrient availability and affecting the structural properties of dissolved organic matter (DOM). For instance, the application of goethite to soil limits P and N bioavailability and increases

the aromatic content of water extractable organic matter (WEOM), which may lower the ratio of fungi to bacteria (Heckman et al., 2012). In general, fungi have low C use efficiency (CUE) (Silva-Sánchez et al., 2019) and are associated with efficient N cycling (Wardle et al., 2004). Unlike bacteria, fungi require less N per unit biomass which may result in decreased N mining from MAOM. In addition, applying goethite together with arbuscular mycorrhizal fungi (AMF) to soil decreased priming in the rhizosphere by protecting OM through sorption and aggregate formation by AMF hyphae (Jeewani et al., 2021b). Godbold et al., (2006) hypothesized that the turnover of the mycorrhizal external mycelium is the dominant process by which root-derived C is incorporated into stable SOM pools (Godbold et al., 2006) and distributed throughout the soil (Frey, 2019), which contribute to SOM sequestration (Godbold et al., 2006). However, mycorrhizal fungi can destabilize SOM by multiple mechanisms summarized in Frey (2019). For example, N in MAOM can be made available to plants by mycorrhizal hyphae which extend plant roots deeper in soils and may destabilize aggregates that protect MAON (Jilling et al., 2018).

7.2 Birch effect

The Birch effect is defined as a short-term pulse in C and N mineralization caused by soil drying and rewetting. Although many studies have been done on N mineralization and nitrification (Birch, 1958, 1959, 1960, 1964; Wilson and Baldwin, 2008), the studies on the Birch effect have mainly focused on C. A pattern has been observed was that N mineralization rate increases as soil becomes drier, along with a rapid decline when soil is rewetted. Soil moisture is accompanied by increased NO_3^- production. The origin of this pattern remains elusive, though the Birch effect is generally tied to multiple interacting mechanisms, including the dissolution of organo-mineral bonds, which increases the accessibility of substrates to microbial degradation.

Wilhelm et al., (2022) investigated the effects of wet-dry cycles on C mineralization of newly added substrates in soils with different Fe and SOC contents and developed under different precipitation regimes. The authors found that wet-dry cycles did not affect C mineralization in the ferrihydrite-rich soil, due to C substrates being

incorporated into microbial biomass and their stabilization in newly formed Fe-organic associations. In contrast, soils with low Fe content did not have enough available surfaces to form Fe-organic associations. Thus, C substrates were more susceptible to mineralization mediated by wet-dry cycles in these soils (Wilhelm et al., 2022). The availability of reactive Fe surfaces in soils can therefore decrease the mineralization of newly formed C during wet-dry cycles.

In tropical regions, soils are widely dominated by Fe oxides that sorb SOC but are also subjected to rapid redox-induced mineral transformations due to highly dynamic wet-dry cycles. In fact, the transformation of amorphous Fe oxides into more crystalline forms decreases soil sorption capacity and nutrient retention (Attygalla et al., 2016; Wilmoth et al., 2018; Chen et al., 2020). We hypothesize that wet dry-cycles can induce rapid electron transfer from and to Fe oxides, known as cryptic Fe cycle, which may affect N bioavailability. During the wet period, Fe(III) oxides can be used as an electron acceptor and be reduced to Fe(II), which can abiotically react with NO_3^- to form NH_4^+, or with nitrite (NO_2^-) to form N_2O. This Fe(II) can be converted back to Fe(III) oxides during the dry period, which may sorb OM and protect it against further degradation or generate oxidative radicals through Fenton reactions that break down organics, including N compounds. This cryptic cycling of Fe will have a varied effect on the role of Fe in controlling N bioavailability over short spatiotemporal scales, which may either increase or decrease bioavailable N. Further research is needed to detangle these interactions.

7.3 Fe in the context of freeze-thaw cycles: the case of permafrost-affected soils

Permafrost-affected soils store large amounts of OC and ON as a result of SOM stabilization due to freezing of SOM and cryoturbation. Along a permafrost soil chronosequence, Joss et al., (2022) found a high percentage of FeOM in cryoturbated soils compared to organic or mineral horizons. Cryoturbation also favors the accumulation of SOM with high C:N ratio at deeper soil depths (Treat et al., 2016a), which also may be present as associations with Fe minerals or in particulate organic matter. Upon thawing, this tremendous amount of SOC and total

nitrogen (TN) facilitate high gross N turnover rates by heterotrophic processes. For instance, Treat et al.(2016b) observed increased nitrogen availability during long thaw seasons in tundra soils, whereas other authors reported higher N_2O emissions from increased denitrification (Cui et al., 2016; Yang et al., 2016; Yang et al., 2018). This is partly because SOC and SOM, previously trapped in FeOM associations, are released and exposed to microbial degradation (Harden et al., 2012; Gentsch et al., 2015; Mueller et al., 2015; Patzner et al., 2020). In fact, Patzner et al., (2020) found that along a thaw gradient, the amount of dissolved organic carbon (DOC) increased as well as the abundance of Fe(III)-reducing bacteria which use Fe(III) as terminal electron acceptor and oxidize OM. The importance of this mechanism in N destabilization likely depends on the extent to which Fe dissolution contributes to soil OM persistence in redox-dynamic permafrost (Patzner et al., 2020). More investigations of Fe control on N bioavailability in permafrost-affected soils are needed, especially with the recent development pointing out that mineral N cycling is as important as ON cycling in the active layers of these soils (Ramm et al., 2022).

8 Impact of global change on Fe-N bioavailability interactions

Global change affects Fe-N interactions in multiple ways. First, climate change is expected to increase the occurrence of the Birch effect as a result of extreme variability in precipitation, which affects N bioavailability. Fe plays multiple roles in this process; Fe can protect ON from decomposition in drier soils but its reaction with light can lead to Fenton-reaction induced ON decomposition (Georgiou et al., 2015). In wetter soil, ON destabilization rates can increase as a result of fluctuations in redox conditions, the occurrence of cryptic Fe cycling and modifications of mineral properties. Second, climate change lead to elevated atmospheric CO_2 concentration (eCO_2), but the effects of the latter on Fe-N bioavailability interactions are not well understood. Recent research showed that eCO_2 stimulates root and microbial respiration, which can decrease soil redox potential causing Fe reduction to proceed (Cheng et al., 2010). The production of Fe(II), which increased by 64%

under eCO$_2$ treatment, caused substantial losses of NH$_4^+$ via Feammox in a 15-year free-air CO$_2$ enrichment (FACE) study in rice paddy systems. Feammox was mediated by autotrophic anaerobes that may use soil CO$_2$ as C source to couple anaerobic ammonium oxidation and Fe reduction (Xu, C. et al., 2020). eCO$_2$ can also increase the destabilization of MAON via priming, as, eCO$_2$ increases root biomass and associated exudate production at deeper soil depths, enabling the liberation of large amount of deep soil N from these associations (Iversen, 2010). This increased turnover of N from MAOM would probably be substantial under future eCO$_2$. Third, land use change involving the conversion to agriculture can decrease SON (García-Oliva et al., 2006). We hypothesize that this decline in SON is influenced by the effects of land use change on Fe cycling. For example, it was observed that the crystallinity of Fe oxides increased when forests were converted to agricultural fields in the Southern Piedmont, USA (Li and Richter, 2012). Additionally, Tan et al., (2019) showed that land use change from fallow to paddy soils promoted Fe reduction by decreasing soil pH and increasing the electron shuttling capacity of SOM due to increased organo-Fe associations (Tan et al., 2019), which may accelerate N turnover by processes such as Feammox. Fourth, freeze-thaw cycles are expected to increase due to climate change. Warmer temperatures increase permafrost thaw which may increase redox-meditated heterotrophic N turnover processes and the destabilization of FeON. To conclude, global change affects the roles of Fe in N bioavailability which may in turn affect the balance between Fe-meditated SON destabilization and protection.

9 Synthesis and outlook

Attempts at understanding controls and drivers of N bioavailability, a fundamental soil ecosystem property, often omit the role of Fe minerals. However, the tendency of proteins to associate strongly with minerals, and the involvement of the latter in both enzymatic and non-enzymatic reactions that influence the N cycle has motivated this review, which specifically focuses on Fe-N bioavailability interactions (Fig. 4). Including Fe in current models of SOM is challenging because the mechanisms by which Fe controls N storage, stabilization, bioavailability, and

loss are complex and remain incompletely understood. This is because the present knowledge is, on one hand, based on OM-mineral correlations, which is a simplistic approach since correlations tend to be specific for certain soil conditions and types (Kleber et al., 2021; Wagai et al., 2020), and on the other hand, knowledge is impeded by limitations in the analytical framework used to explore these interactions. In this section, we highlight challenges and opportunities for future research.
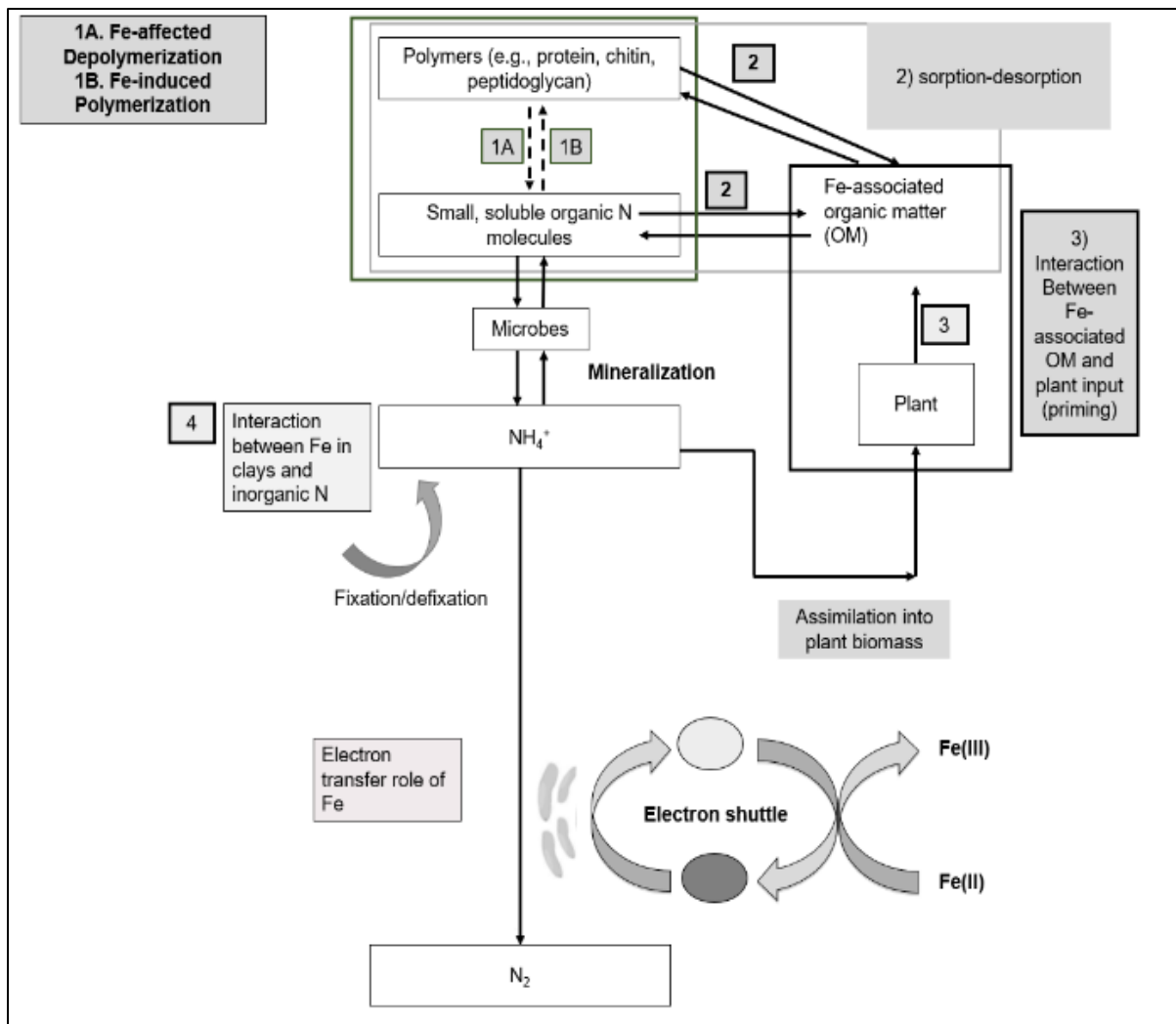
Figure 4. Fe affects N bioavailability in soils. This Fig. doesn't specify soil conditions under which an Fe role may proceed.

9.1 Sorbent role of Fe in controlling N bioavailability

The sorbent role of Fe in controlling N bioavailability is multifaceted. Sorption can protect N from decomposition

by reducing the activity of enzymes and limiting the accessibility of N substrates to degradation mechanisms.

However, a fraction of sorbed N is bioavailable (Bird et al., 2002; Kleber et al., 2007), or can be made available

by processes such as priming or displacement by competitive organics. Thus, the concept of "sorptive stabilization" of N substrates does not stand as a conclusive explanation for N persistence in soils and should rather be revisited. In this context, sorption to Fe minerals may impose spatial constraints on the accessibility of N substances to microbes, as sorption can locate N in physically isolated spaces such as micropores, microaggregates, or microdomains of densely arranged clays which slows down its decomposition and decreases its bioavailability (Kleber et al., 2021).

Research on Fe-meditated N depolymerization has mostly focused on proteins (Wanek et al., 2010; Noll et al., 2019; Reuter et al., 2020), since proteins alone constitute 60% or more of the N in plant and microbial cells (Fuchs, 1999) and are strongly sorbed to Fe surfaces. However, not all soil and mineral-associated N is protein. Rather, N exists in a variety of chemical forms (box 1) including microbial cell wall compounds. Using Fourier transform infrared spectroscopy (FTIR) and isotope pool dilution (IPD), multiple studies have shown the importance of microbial cell wall depolymerization in the delivery of soil N (Hu et al., 2017; Hu et al., 2018; Hu et al., 2020). In addition, depolymerization of membrane lipids and nucleic acids is not yet characterized despite the detection of their degradation products in soils (Warren, 2021). This leads to the following question: how important is the chemical form of Fe-associated N in determining soil N bioavailability? This is relevant since the molecular characteristic of different N forms influences the type and strength of bonding with minerals, which may affect N bioavailability. For instance, Fe oxyhydroxides binds amino sugars more strongly than proteins in boreal forests (Keiluweit and Kuyper, 2020), likely allowing less mineralization from the former compared to the latter compounds.

9.2 Structural role of Fe in controlling N bioavailability

Despite a small number of studies relating structural Fe in clays and aggregates to N bioavailability, the dynamics of these interactions and relevant mechanisms remain elusive. Several questions remain to be resolved, including:

are the original structure and physico-chemical characteristics of clay minerals restored upon reoxidation of its structural Fe? If so, what are the implications for NH_4^+ release and fixation and other processes that influence loss and gain of bioavailable N? How relevant is the loss of Fe by solubilization and reduction to microaggregate instability and N bioavailability in soils? In addition, the relevance and the occurrence of Fe-induced C and N polymerization is soils awaits confirmation, because this phenomenon has been observed only in laboratory settings.

9.3 The role of Fe as a catalyst in controlling N bioavailability

Assessing the importance of Fe-meditated ROS generation in N bioavailability is a formidable challenge. In fact, despite being common in soils, ROS have extremely short lifetimes and are highly reactive towards other soil constituents such as carbonates and bromide (Kleber et al., 2021), which complicate their detection in soils. They are produced by both abiotic and biotic pathways, and the contribution of each pathway to N bioavailability remains elusive. Additionally, rates and mechanisms of ROS production from these two pathways are still not known. Such information is particularly important to understand N dynamics in environments conducive to ROS formation, such as oxic/anoxic zones, environments with intense solar radiation or in boreal forests where fungi use ROS based mechanisms to access Fe-sorbed N. In contrast to their decomposition role, Yu et al.(2020) found an important role of Fe-meditated ROS production in OM polymerization, which increases the recalcitrance of OM and its resistance to degradation mechanisms (Yu et al., 2020). This finding sheds light on other controls and pathways relevant to N bioavailability. For example, under what conditions can the role of Fe-mediated ROS generation on N bioavailability be shifted from decomposition to protection? And how will this evolve in a changing world where solar radiation is becoming more intense and the frequencies of extreme events (e.g., droughts, rain) is increasing?

9.4 Electron transfer role of Fe in controlling N bioavailability

The capacity of Fe to act as an electron acceptor and donor can affect bioavailable N loss from soils by processes such as Feammox and denitrification. To further understand these processes, more research is needed on cryptic Fe cycling and the controls over the oxidation-reduction dynamics of Fe in soil, since preservation of oxidized Fe promotes N stabilization within mineral associations. For instance, the effects of added electron shuttles on the extent and the rate of Fe(III) reduction and associated loss of N via Feammox have been investigated, however, the capacity of SOM and organo-Fe associations to transfer electrons has received less attention (Sposito, 2011; Xu, Z. et al., 2020). The characterization and mapping of spatiotemporal redox heterogeneity also deserves attention (Wilmoth, 2021).

9.5 Varied analytical approach is needed to characterize Fe-N interactions

To understand the roles of Fe in controlling N bioavailability, a varied analytical approach must be adopted to enable a more holistic and multidimensional view of these interactions, considering all the possible outcomes of Fe reactions on N as driven by the physico-chemical and biological characteristics of soil and management. This approach is essential to provide realistic turnover rates of N and decipher the underlying mechanisms of Fe-N reactions in soil, in contrast to controlled lab experiments which do not represent soil in its complexity and heterogeneity. This approach should also capture variations in the processes of interest within multiscale and time dimensions. Here, we present most common and powerful techniques that can be combined in the framework of this varied approach to understand Fe-N interactions. Note that an extensive list of techniques is out of the scope of this review.

  (a) Imaging techniques: Techniques such as Synchrotron XAS and Synchrotron X-ray allow the
       identification and the characterization of structural and chemical properties of minerals as well as their

oxidation states. They can also be used to determine the speciation of SON and dissolved organic nitrogen (DON) as well as the structural characteristics of soil, such as pore size and pore connectivity. These information help, for example, to characterize the fine-scale redox heterogeneity (Wilmoth, 2021) that affects Fe cycling and its interconnection with N bioavailability. In addition, these techniques are used to observe and investigate the 3D structure of organo-Fe minerals in soils. Kleber et al. (2021) called for using them in studies of enzyme activity because they allow the investigation of the natural structure of organo-mineral associations without alteration (Kleber et al., 2021). However, while using advanced imaging techniques reveals information at fine scales, upscaling such data is challenging (Wagai et al., 2020).

(b) Microbial techniques: They provide information on the identity of microbial taxa regulating soil biogeochemical processes in question. They include techniques such as metatranscriptomics which can be used to distinguish the biological from the abiotic pathways used to direct redox reactions (Wilmoth, 2021), and metagenomics that were used recently to explore coupled nutrients interactions, including coupled Fe-N reactions (Ma et al., 2021).

(c) Isotope techniques: Isotopes can be used to determine gross rates and the investigation of the pathways and mechanisms of the processes in question. They can also be used to determine OM pools with varying turnover rates. Stable isotope probing, which is a high-resolution technique, can also be used to trace the microbial uptake of N as affected by Fe minerals as well as its fate in soil environments.

(d) Molecular characterization techniques: These techniques, which include FTIR, allow the identification of different soil organic molecules and the analysis of their bonding mode and strength with minerals.

9.6 Concluding Comment

As a final commentary on Fe-N bioavailability interactions, we propose the following questions: how much N can be mobilized by Fe-related mechanisms? What are the controls on these interactions? And how important are certain mechanisms relative to others in securing N bioavailability in the context of global change? Do reactions observed in laboratory settings occur naturally in soils? We also urge the field to develop new methods and techniques, such as those capable of detecting low concentrations of ROS and their fate in soil environment, or the products of mineral-induced OM polymerization.

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CHAPTER II: Iron-organic carbon coprecipitates reduce nitrification by restricting molybdenum availability in soils

Abstract. Nitrification, an important pathway of nitrogen transformation in soils, converts ammonium (NH_4^+) to nitrate (NO_3^-) using metalloenzymes, the activity of which depends on iron (Fe), molybdenum (Mo), and copper (Cu) availability. Iron (Fe)-organic carbon coprecipitates (or Fe-OC flocs) are components of soil that may affect nitrification by changing the bioavailability of these metals. Here, we used flocs of different chemistry (aromatic and aliphatic) and known Fe and C composition to investigate their effects on nitrification in soils along a soil C gradient. Both aromatic and aliphatic flocs reduced net nitrification, but the magnitude of their effect was more pronounced in mineral than in organic soils. Both flocs reduced net nitrification similarly in each soil. In the presence of floc, the bioavailability of Mo (assessed by changes in the concentration of water-soluble Mo) was dramatically decreased in mineral soils, possibly because Mo was incorporated into or adsorbed to flocs or their decomposition products. In contrast, Mo bioavailability in organic soils was decreased to a lesser extent by flocs, likely because organic matter limited floc adsorption capacity and/or released Mo through mineralization. The depletion of bioavailable Mo by flocs in agricultural soils has the potential to impede soil nitrification and extend the duration of NH_4^+ and its availability to plants and microbes.

1 Introduction

Nitrification, an important pathway of N transformation in soils, converts NH_4^+ to NO_3^- via intermediates such as nitrite (NO_2^-) and hydroxylamine (NH_2OH). Although there have been several studies addressing the role of Fe in nitrification (Butler et al., 1986) the effects of Fe bound to organic carbon (OC) are not well characterized. However, Fe-bound OC contributes up to 38% of soil total OC (in some soils) as a result of adsorption and

coprecipitation processes (Zhao et al., 2016). The latter process is ubiquitous in soils and sediments experiencing significant changes in pH or redox conditions and the resulting Fe-OC coprecipitates (or Fe-OC flocs) are critical to stabilizing C against microbial decay and determining Fe reactivity, which may limit its participation in nitrification. Nevertheless, the stability of these flocs, defined here as resistance to both microbial and chemical degradation, can be influenced by OC chemical composition as well as soil conditions and properties. First, the chemical composition of OC influences the binding strength with mineral surfaces (Newcomb et al., 2017). High-molecular-weight OC containing abundant functional groups is preferentially adsorbed by either strong or irreversible processes (Lehmann et al., 1987; McBride, 1987) leading to highly stable flocs. In contrast, aliphatic compounds such as carbohydrates are biodegradable and yield less stable flocs. Second, changes in soil pH or redox conditions can compromise the stability of Fe-OC flocs such as by inducing the reductive dissolution of Fe and the subsequent release of OC. Adhikari and Yang, (2015) found that despite the preferential adsorption of aromatic compounds, aliphatic compounds were stabilized during the reductive dissolution of hematite(Adhikari and Yang, 2015), which contradicts the observations of Kalbitz et al., (2003). Third, clays present in soil can stabilize flocs against microbial decay (You et al., 2022). Therefore, OC chemistry as well as soil properties and conditions are expected to determine floc stability and reactivity; the more stable a floc is, the less influence it is expected to have towards nitrification.

Beyond controlling Fe and OC availability, flocs may impede nitrification by controlling the availability of trace metals and nutrients that are required for microbial growth and metabolism. Nitrification is a three-step process, and each step is regulated by different metalloenzymes (Lancaster et al., 2018). Nitrification is initiated by the activity of Ammonia Monooxygenase (AMO), which hydrolyzes ammonia (NH_3) to NH_2OH. In addition to oxygen, this step is believed to be dependent on copper (Cu), an essential cofactor and catalyst in AMO (Ensign et al., 1971). In the second step, NH_2OH is oxidized to NO_2^- by the activity of hydroxylamine oxidoreductase (HAO) or to nitrous oxide (N_2O) by Heme P460 cofactors in a process that utilizes Fe (Lancaster et al., 2018).

The final step is the oxidation of NO_2^- to NO_3^- by nitrite oxidoreductase (NXR). This enzyme hosts a molybdenum (Mo) cofactor (Moco) which binds the substrate molecule (NO_2^-) (Chicano et al., 2021) and contains electron transfer cofactors, namely iron sulfur clusters, which may transfer electrons from NO_2^- oxidation (Lancaster et al., 2018). Despite their importance in the nitrification process, metals can inhibit microbial activity beyond safe thresholds.

Few studies have considered the effects of Fe-OC flocs on metal bioavailability in soils, presumably due to their high stability in soil environments. However, interactions with OM and the highly sorptive Fe minerals can control metal bioavailability. For instance, Seda (2014) showed that Fe and OM coprecipitates increase the retention of Cu in soils, but to a lesser extent than Fe and OM adsorption complexes. Therefore, the purpose of this study is to explore the effects of Fe-OC flocs on nitrification in soils with different properties, such as C, N, and clay content. To do so, we created model Fe-OC flocs of known Fe and C composition (aromatic and aliphatic C) to understand how flocs affect nitrification in soils. Our hypothesizes are:

(a) Hypothesis 1: Fe-OC flocs reduce nitrification. Aliphatic floc reduce nitrification more than aromatic floc because it is less stable and more reactive.

(b) Hypothesis 2: The effect of Fe-OC flocs on nitrification are less pronounced in organic relative to mineral soils.

(c) Hypothesis 3: Fe-OC flocs or their decomposition products decrease the bioavailability of metal cofactors Mo, Fe and Cu necessary for nitrification.

2 Materials and methods

2.1 Preparation of Fe-C flocs

All chemicals used were high purity and purchased from Sigma-Aldrich. Tannic acid (TA) and carboxymethyl cellulose (CMC) were used to prepare the aromatic and aliphatic Fe-OC flocs, respectively. To prepare the aromatic floc, 2 % (w/v) TA solution and 2 % (w/v) iron (III) sulfate (FeS) solution were prepared using deionized water (DI). In an Erlenmeyer flask, 18 mL FeS solution were added to 10 mL TA solution under vigorous stirring, and the pH was adjusted to 7 with 1 M NaOH. The resulting black solution was centrifuged at 10000 rpm for 15 min, after which the supernatant was discarded, and the precipitate (floc) was washed 3 times with DI water to remove excess Fe. The floc was oven-dried overnight at 30^0 C and ground using a pestle and mortar to obtain a fine powder. Powdered flocs were stored at room temperature in a closed container. To prepare the aliphatic floc, 3 mL of 2 % FeS solution was added to 10 mL of a 2 % (v/v) CMC solution. The resulting solution was shaken manually and centrifuged at 10000 rpm for 15 min, after which the supernatant was discarded, and the floc was washed 3 times with DI water. The floc was freeze-dried and ground to a fine powder before storing   at room temperature in a closed container.

2.2 Characterization of Fe-C flocs composition

Total C and N analyses were performed on powdered Fe-OC flocs using an elemental analyzer (EAS 4010, Costech Analytical Technologies Inc., Valencia, CA). Floc Fe content was determined by digesting 30 mg of floc in 1 mL nitric acid HNO_3 (69 %, 16 M) followed by determination of dissolved Fe with a ferrozine-based colorimetric assay. The results of these analyses are presented in Table 1.

Table 1: C, N and Fe content of floc (means ± standard error, n = 3)

Floc	C (mg.kg^{-1})	N (mg.kg^{-1})	Fe (mg.g^{-1})
Aromatic floc	22.51 ± 0.37	ND*	1.4 ±0.31
Aliphatic floc	27.36 ± 0.08	ND*	0.26 ±0.04

*ND: Not detectable

2.3 Soil preparation

Soils along a C gradient (1% C Sand, 3%, 11% and 16 %) were collected from the top 0-15 cm of Twitchell Island, located on the western portion of the San Francisco Bay Delta (California, USA). An additional 1% C soil was collected from Russell Ranch at the University of California at Davis. Most soil from Twitchell island is a Rindge mucky silt loam (Euic, thermic Typic Haplosaprist), formed from Tule and reed deposition. The Twitchell Island 1% C soil has a sandy texture, whereas Russell Ranch soil is a clay (1%C CL). For each soil, five samples were randomly collected, composited, and sieved (2 mm). Visible plant residues and roots were removed and soils were air-dried. Gravimetric water content (GWC) (105°C for 48–120 h) and Water Holding Capacity (WHC) of soils were determined. The percentages of clay, silt and sand were determined by hydrometer method, with a pre-treatment with H_2O_2 to remove OM. pH was determined in 1:5 soil: water slurries. Selected soil properties and initial metal concentrations are presented in Tables 2 and 3, respectively.

Table 2: Selected properties of soils (means ± standard error, n = 4 where indicated)

Soil	C (%)	N (%)	C:N ratio	Sand (%)	Clay (%)	Silt (%)	pH
16% C	16 ± 0.20	1.12 ± 0.01	14.73 ± 0.08	12.8	19.4	67.8	6.1 ± 0.00
11% C	11 ± 0.28	0.68 ± 0.00	13.94 ± 0.17	12.4	24.2	63.4	5.9 ± 0.02
3% C	3 ± 0.06	0.28 ± 0.03	10.7 ± 0.05	5.4	32.4	62.3	6.6 ± 0.07
1% C- CL	1 ± 0.03	0.13 ± 0.02	8.53 ± 0.01	20.5	46.7	32.8	7.8 ± 0.02
1%C- Sand	1 ± 0.04	0.08 ± 0.00	13.13 ± 0.43	91.7	7.0	1.3	6.5 ± 0.02

Table 3: Initial metal and DOC concentrations in soils (means ± standard error, n = 4)

Soil	Total dissolved Fe (mg. kg^{-1})	Water-soluble Cu (mg. kg^{-1})	Water-soluble Mo (mg. kg^{-1})	DOC (mg. kg^{-1})
16% C	4.38 ± 0.42	0.053±0.003	0.025±0.001	837.43 ± 3.27
11% C	1.29 ± 0.05	0.042±0.0006	0.017±0.0001	391.21 ± 2.47
3% C	0.16 ± 0.00	0.048±0.014	0.009±0.01	139.26 ± 0.85
1% C- CL	0.61 ± 0.00	0.052±0.01	0.014±0.009	73.09 ± 4.97
1%C- Sand	3.42 ± 0.02	0.027±0.002	0.0031±8.49E-05	239.6 ± 0.79

2.4 Soil incubations

2.4.1 Main incubation

Flocs were applied to 6 g air-dried soil at a rate providing 2 mg C/g soil. Flocs were thoroughly mixed with soils and incubated at 65 % of WHC at 30°C for 30 days. Control samples were prepared by incubating soil without flocs. Each treatment was replicated four times. Soil-floc mixtures were placed in specimen cups, which were then placed in a mason jar containing 2 ml of DI water to maintain moisture. The jars were closed with a foam lid to allow gas exchange. NH_4^+, NO_3^-, total dissolved Fe, dissolved organic C (DOC) were analyzed on days 0, 3, 5, 7, 10, 17 and 30 of the incubation. Net N mineralization and nitrification (mg N kg^{-1} dry soil) in each treatment

was calculated as the difference between the final and initial NH_4^+ and NO_3^--N contents, respectively. Reduction in net nitrification (Rednit) was calculated as the percent difference between NO_3^--N contents in treatments and controls (equation1). We also measured NO_2^- concentrations but only in 1% C Sand, 1% C CL and 11% C soils. Hot water extraction of Mo and Cu was performed on samples incubated for 30 days at 30°C: 20 mL of DI were added to samples and placed in a hot water bath for 1 h with occasional shaking. Then, samples were shaken for 1 h, centrifuged (10.000 g for 10 min) and passed through 0.45-µm syringe filter to remove particulate material. Dilution with 3% nitric acid (HNO_3) followed (the dilution factor was 1.03), after which samples were analyzed by Inductively Coupled Plasma (ICP) at the ICP laboratory at the University of California at Davis.

$$\text{Equation 1: } \% \text{ Rednit} = ((NO_3^- \text{-} N_{treatment} - NO_3^- \text{-} N_{control}) / NO_3^- \text{-} N_{control})) \times 100$$

2.4.2 Additional incubation

To confirm our hypothesis 3, we performed a laboratory incubation to test the effects of added metal cofactor on Rednit. Flocs were applied to 4 g of 1% C Sand receiving 10, 50 and 100 mg kg^{-1} Mo as sodium molybdate. Control samples were prepared by incubating soil with flocs, but without the addition of Mo. We measured net nitrification (mg N kg^{-1} dry soil) as the difference between the final and initial NO_3^--N contents in each treatment.

2.5 Characterization of Fe-OC flocs stability

To characterize Fe-OC floc stability, we combined thermal stability analyses, which serves as a proxy of binding strength between the mineral and the organic fractions, with stability assessments in incubation experiments, measured by the amount of Fe and OC released from flocs. Thermal stability was measured by thermal gravimetric analysis (TGA) using an SDT Q600 V20.9 Build 20 and was performed under N_2 purge (20 mL/min) from 25 to 1500 ^{0}C with temperature increments of 20 ^{0}C/min.

2.6 Statistical analyses

All experiments were evaluated using Excel and R statistical software (version 3.6.2). We performed analysis of variance (ANOVA), regression analysis, and principal component analysis (PCA) solely on data from day 30 of the incubation.

3 Results

3.1 Thermal and biological stability of Fe-OM flocs

Our results for thermal stability analysis showed that TGA-T_{50} of the aromatic floc, which is the temperature at which half of the OC is removed, is greater than that of the aliphatic floc (Fig. 5). Thus, to remove the same fraction of C mass, the aromatic floc required higher temperature (1100 ^{0}C) than the aliphatic floc (380 ^{0}C), which indicates that the former is more thermally stable. These results are in general agreement with stability assessments of Fe-OC floc in the main incubation experiment, since soluble Fe and OC increased in all soils treated with the aliphatic floc but not with the aromatic floc- assuming the soluble Fe and DOC originate from floc decomposition (Fig. 6).
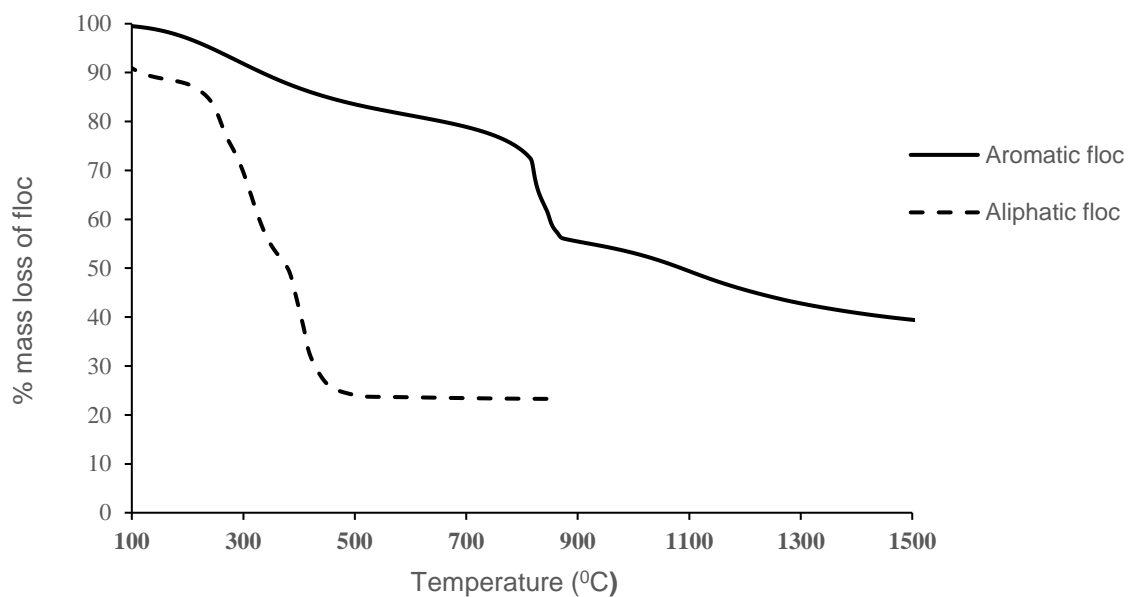
Figure 5. Thermal stability of the aromatic and aliphatic flocs

The pattern and the amount of Fe and DOC released from the aliphatic floc differed in each soil (Fig. 6). At day 30, soluble Fe and OC concentrations in 1% C- Sand and 16% C soils exceeded those of the other soils. Of all the soils, Fe and DOC were correlated (positively) only in 1% C Sand ($r^2 = 0.6$). The release of 10% and 2% of floc C in the 3% C and 1% C CL soils, respectively, was not accompanied by increased Fe, which remained unchanged throughout the incubation period.
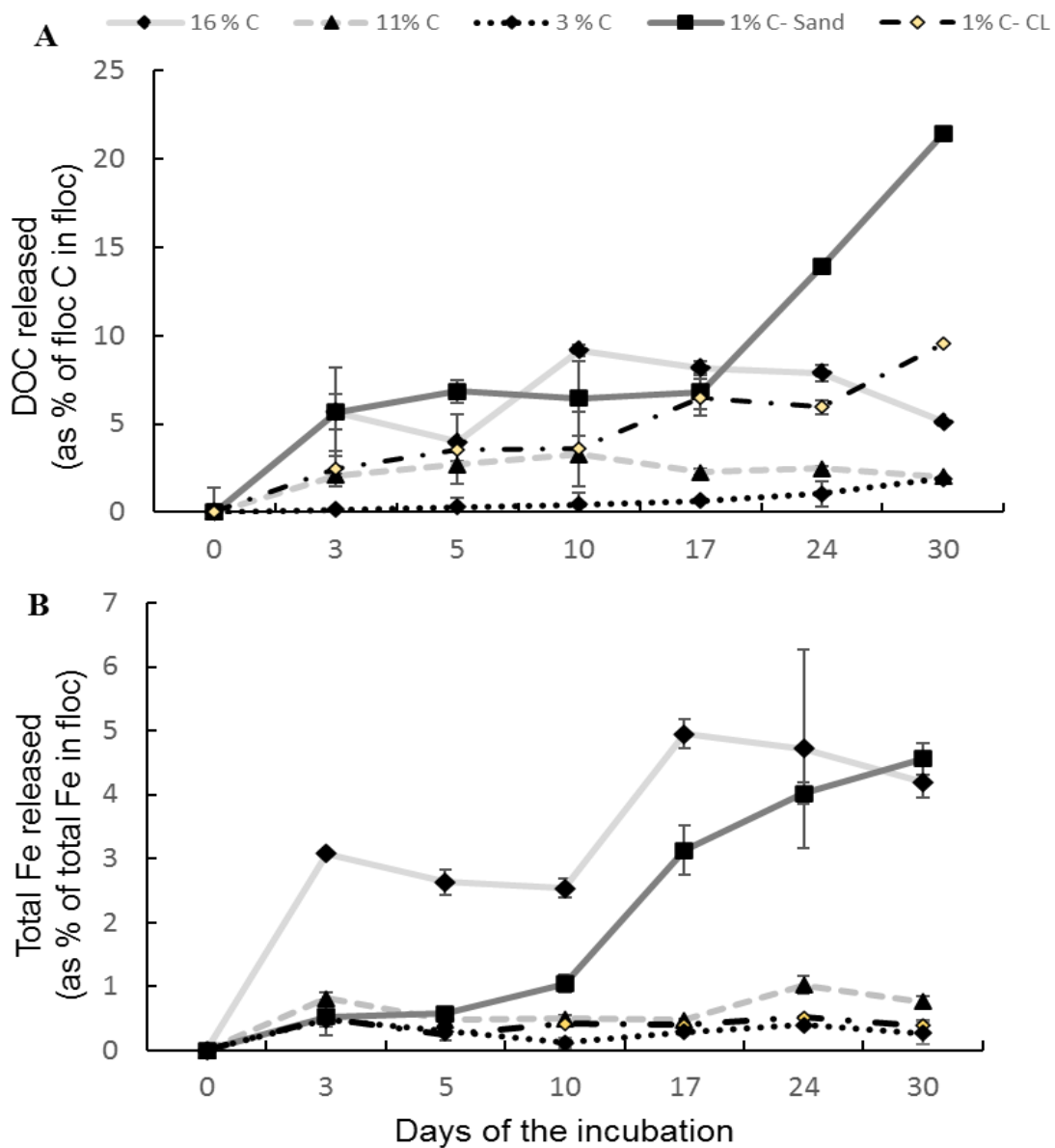
Figure 6. Percent DOC (A) and Fe (B) released from the aliphatic floc in soils in the main incubation experiment.

3.2 Flocs reduced net nitrification more in mineral than in organic soils

We measured net nitrification and calculated Rednit in soils amended with both floc types (Fig. 7: A, B). A two-way ANOVA was performed to test the main and interaction effects of floc and soil types on Rednit (Table 4). Both flocs significantly reduced net nitrification in all soils. The interaction between soil and floc type was also significant, suggesting that the ability of floc to affect nitrification depends on soil type. As such, the magnitude of floc effects on nitrification was more pronounced in mineral (< 3% C) than in organic soils (> 3% C). For instance, Rednit was 50% in 1% C Sand compared to 20% in 16% C soil with both flocs. Within the same soil, both flocs reduced net nitrification similarly (p= 0.33, F=0.97). As assessed by the F value, the main effect of floc type was larger than that of soil type and interaction effect.

Table 4: ANOVA results. Levels of significance: p< 0.05 (*), p<0.01 (**) and p<0.001 (***). NS: not significant

	Rednit	NO_2^- concentration	Water-soluble Mo	Water-soluble Cu	pH
Soil type	F=132.12 p< 2e-16 ***	F=94.55 p= 6.39e-13***	F=1260.64 p< 2e-16	F=27.55*** p=1.35E-11	F=225.25 p< 2e-16 ***
Floc type	F=60.97 p=1.4e-14***	F=21.29 p=2.81e-06***	F=102.83 p < 2e-16***	F=6.72 0.0027*	F=59.01 p=2.64e-13 ***
Interaction effect	F=10.589 p=0.0001***	F=7.34 p=0.0003***	F=11.03 p=1.87e-08***	F=1.77 p=0.10 NS	F=12.50 p=3.14e-09 ***
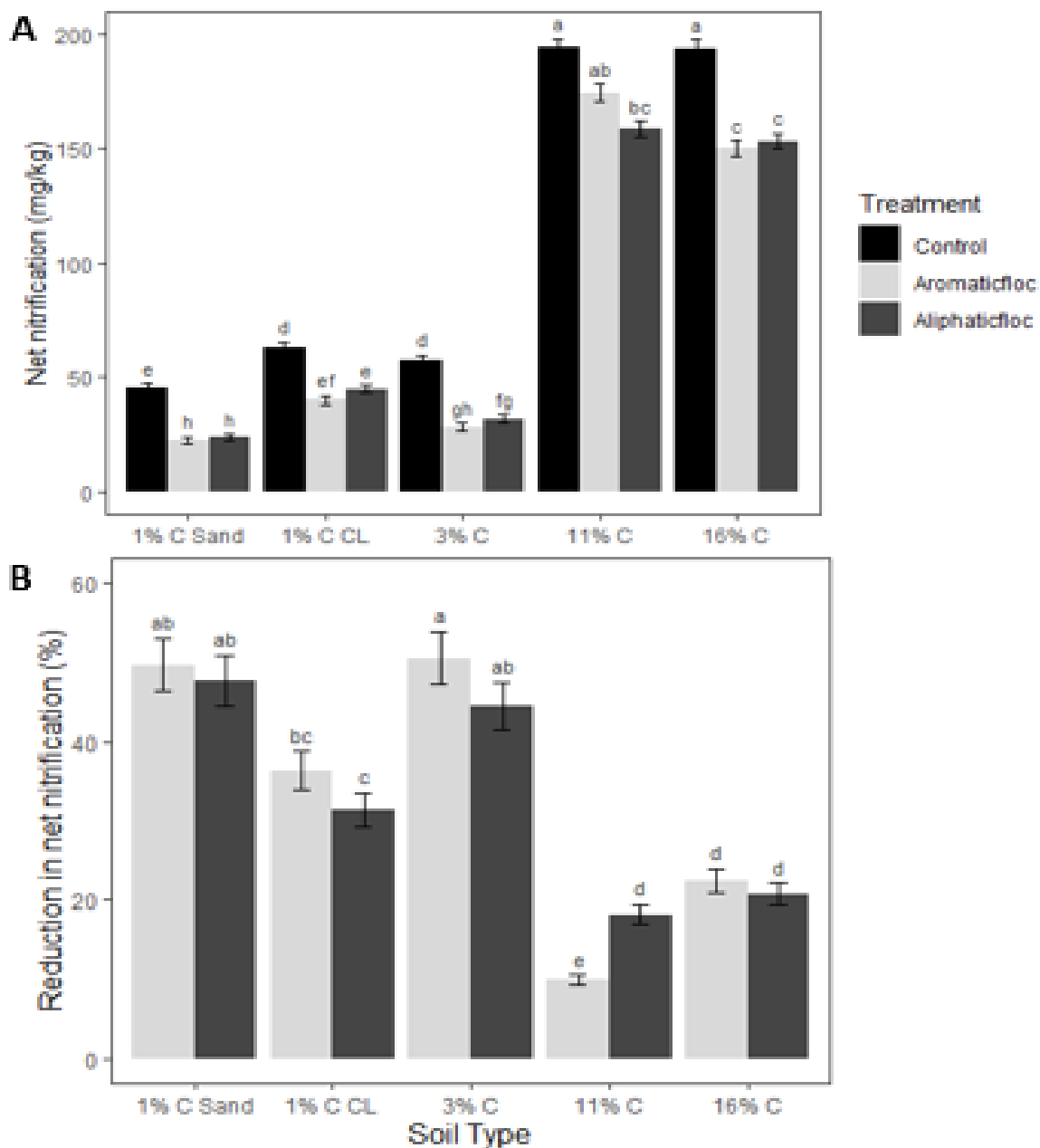
Figure 7. Net nitrification (A) and Rednit (B) in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment.

3.3 Rednit was accompanied by changes in pH, water-soluble Mo, water-soluble Cu and NO_2^- concentrations in soils

We also performed a two-way ANOVA to test the main and interaction effects of soil and floc type (Table 4) on NO_2^-, water-soluble Mo, water-soluble Cu concentrations, and pH, for which data are presented in Figures 8, 9, and 10 and 11, respectively. Some generalizations can be made: first, main effect analysis showed that both flocs significantly affected pH, NO_2^- and water-soluble metal concentrations, more so in mineral than in organic soils. Second, with some exceptions, floc effect was more pronounced with the aliphatic compared to the aromatic floc. Third, the main effect of soil type was larger than that of floc type and interaction effects (Table 4), indicating the strong influence of the soil type on pH, NO_2^- and water-soluble metals. Indeed, mineral soils were more responsive to floc input than organic soils. Fourth, the interaction between floc and soil type was significant except for water-soluble Cu. Fifth, both flocs reduced water-soluble Mo and pH, but had the opposite effect on water-soluble Cu. For instance, in the 1% C CL soil, water-soluble Mo was reduced by 42.2% and 62.6%, whereas water-soluble Cu was increased by 40.3% and 90.2%, respectively, in aromatic and aliphatic floc treatments. Besides this, flocs caused the accumulation of NO_2^- in 1% C Sand and 1% C CL treated with flocs compared to control soils, however, NO_2^- accumulation was minimal in 11% C soil.
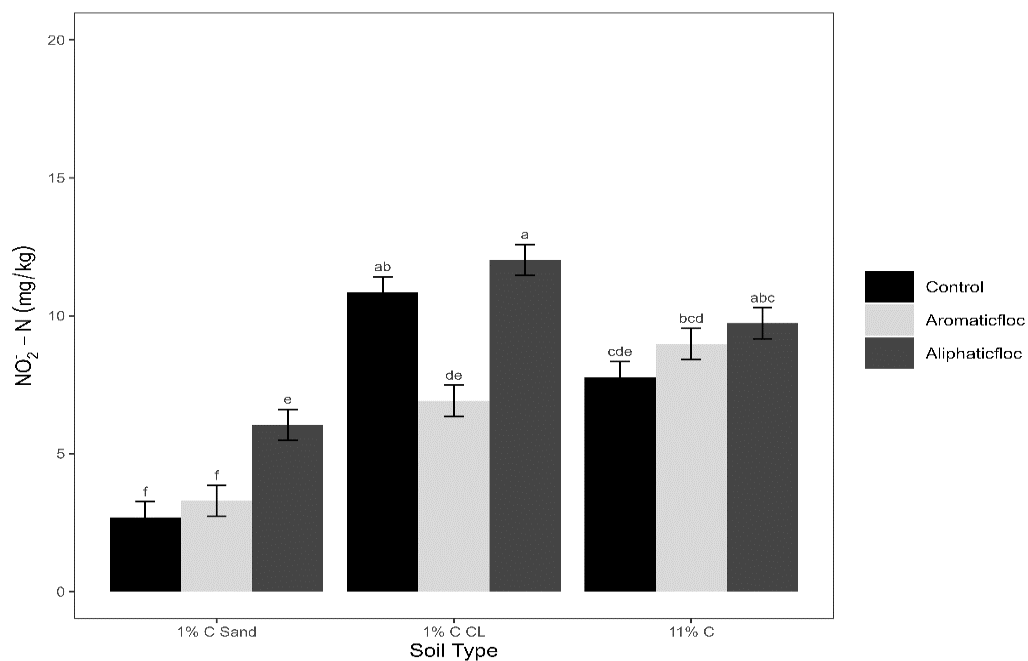
Figure 8. NO$_2$-N concentrations in soils amended with flocs.
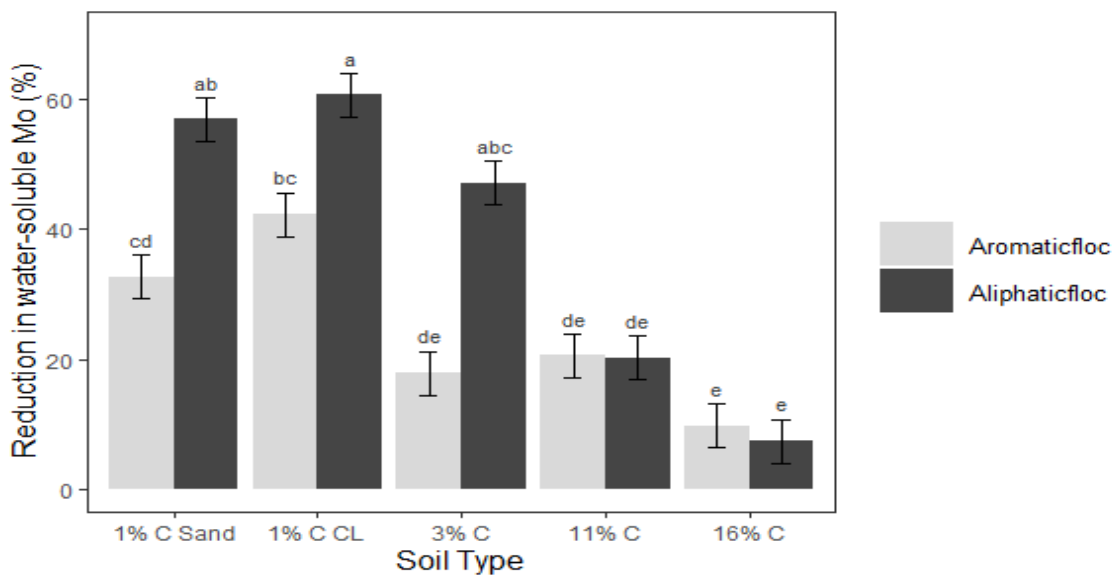
Figure 9. Reduction in water-soluble Mo in soils amended with aromatic and aliphatic flocs, measured at day 30 of the incubation. Values are averages of 4 repetitions per treatment.
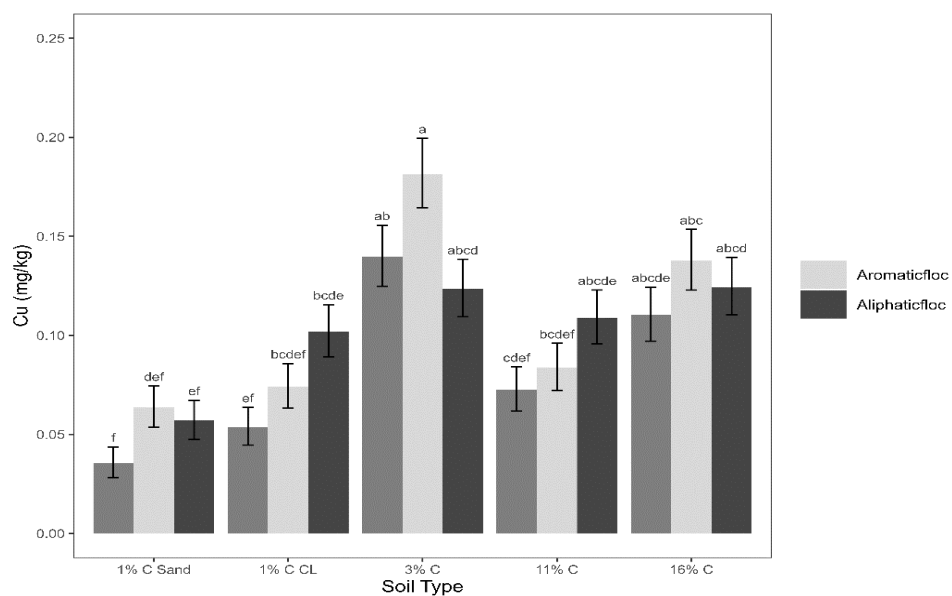
Figure 10. Water-soluble Cu in soils amended with aromatic and aliphatic flocs at day 30 of the incubation. Values are average of 4 repetitions per treatment.
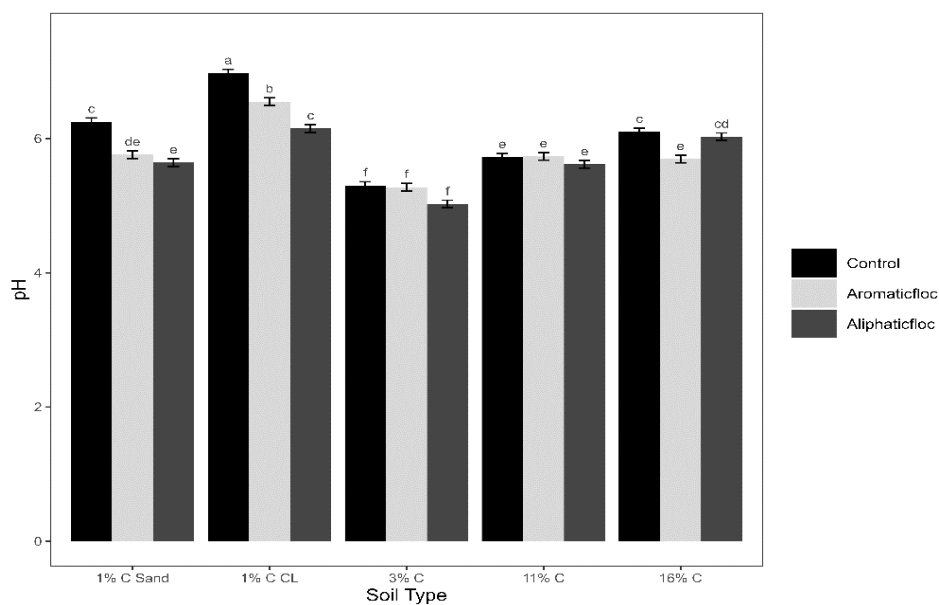
Figure 11. Reduction in pH in soils amended with aromatic and aliphatic flocs, measured at day 30 of the incubation. Values are average of 4 repetitions per treatment.

3.4 Relationships and sources of variation in Rednit data

We conducted Principal Component Analysis (Fig. 12) to explore relationships among Rednit, soil properties and soluble metal concentrations. The first principal component (PC1) accounted for 42.1% of the variability and the most important eigenvectors were water-soluble Mo, soil total C, and N content. These factors had approximately equal negative loadings (Supplemental Table S6). The second Principal Component (PC2) accounted for 21.8% of the variability and was highly loaded for water-soluble Cu, pH, and sand. The third principal component (PC3) accounted for 18% and the most important eigenvectors were DOC, Fe, and pH. It is noted that the amount of Fe originating for soil was negligible. The fourth and fifth principal components (PC4 and PC5), which accounted for 9 and 6% respectively, were highly loaded for pH and C:N ratio for the former component, and clay+ silt content for the latter component (Supplemental Table S6).

3.5 Relationships between Rednit, soil properties and water-soluble metal concentrations in soils amended with flocs

We used regression analysis to explore relationships between Rednit, water-soluble metal concentrations, soil total C, soil total N, clay + silt content, and pH at 3 levels of study: global (across all soils), soil category (mineral vs. organic soils) and in individual soils. Among these factors, only water-soluble Mo, soil total C and N and clay + silt content were significantly correlated with Rednit (Fig. 13). These relationships were stronger in organic than in mineral soils, as indicated by adjusted r^2 (Table 5). We also noted a strong and significant correlation between Rednit and water-soluble Cu in organic but not in mineral soils and this relationship did not appear at the global level of study.
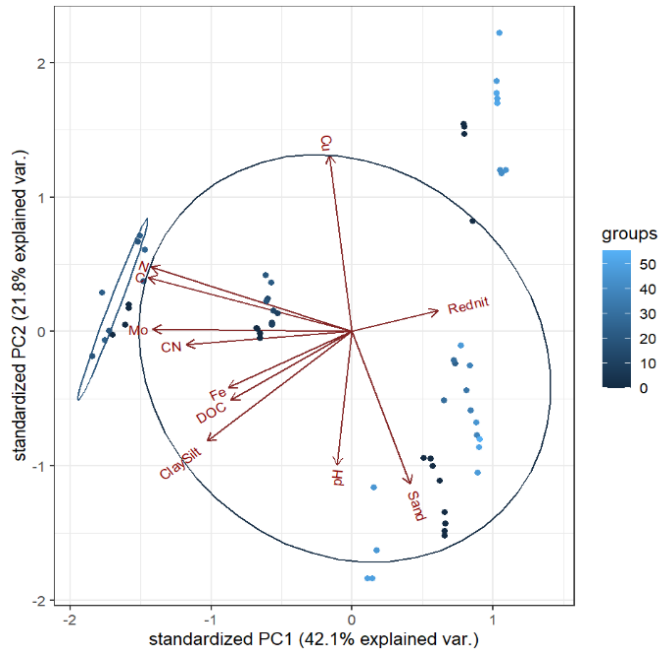
Figure 12. Principal component analysis of factors affecting Rednit.
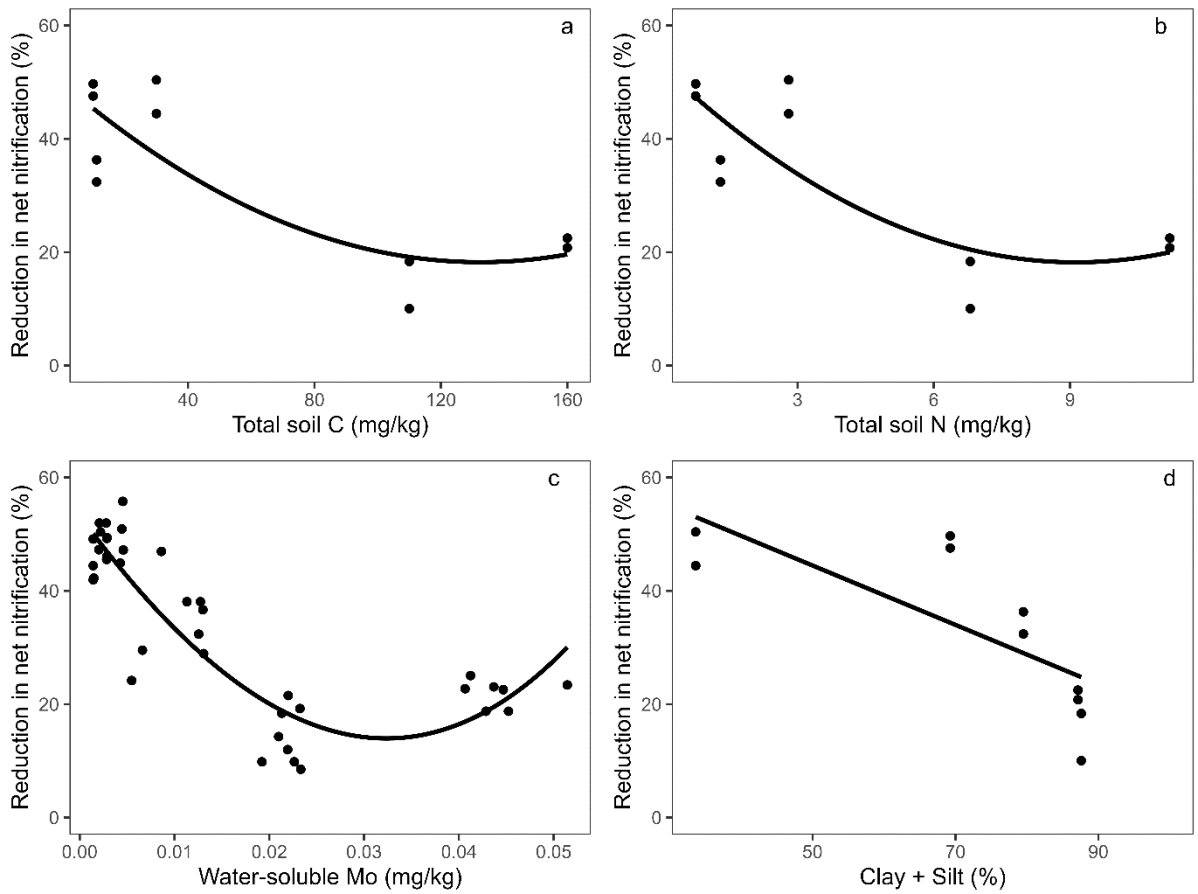
Figure 13. Relationship between Rednit and (a) soil C content, (b) soil N content, (c) water-soluble Mo and d) clay + silt content in soils amended with flocs.

Table 5: Results of the regression analysis between Rednit, water-soluble metals, clay+ silt content and soil total C and N contents on the basis of soil type

Variable	Level of study	Slope	Intercept	Adjusted r^2	p-value
Water-soluble Mo	Organic soils	323.33	7.26	0.46	0.002
	Mineral soils	-1217.7	49.90	0.38	1.05E-16
Water-soluble Cu	Organic soils	0.0035	0.051	0.70	3.1E-05***
	Mineral soils	0.0013	0.045	-0.00058	0.33 NS
Soil total C	Organic soils	3.40	73.98	0.47	0.002***
	Mineral soil	0.40	-0.91	0.079	0.097 NS
Soil total N	Organic soils	0.03	0.36	0.47	0.002***
	Mineral soils	0.001	0.093	-0.022	0.49 NS
Clay + silt	Organic soils	-16.32	1444.68	0.47	0.0019***
fraction	Mineral soils	-0.20	55.74	0.20	0.014618

NS: non-significant.

Of all the studied factors, Rednit was only correlated with water-soluble Mo in individual mineral soils, but not in all individual organic soils (Fig. 14). This correlation was observed only when the control treatment was included in the regression analysis, indicating the possible existence of a threshold concentration under which water-soluble Mo did not affect nitrification.
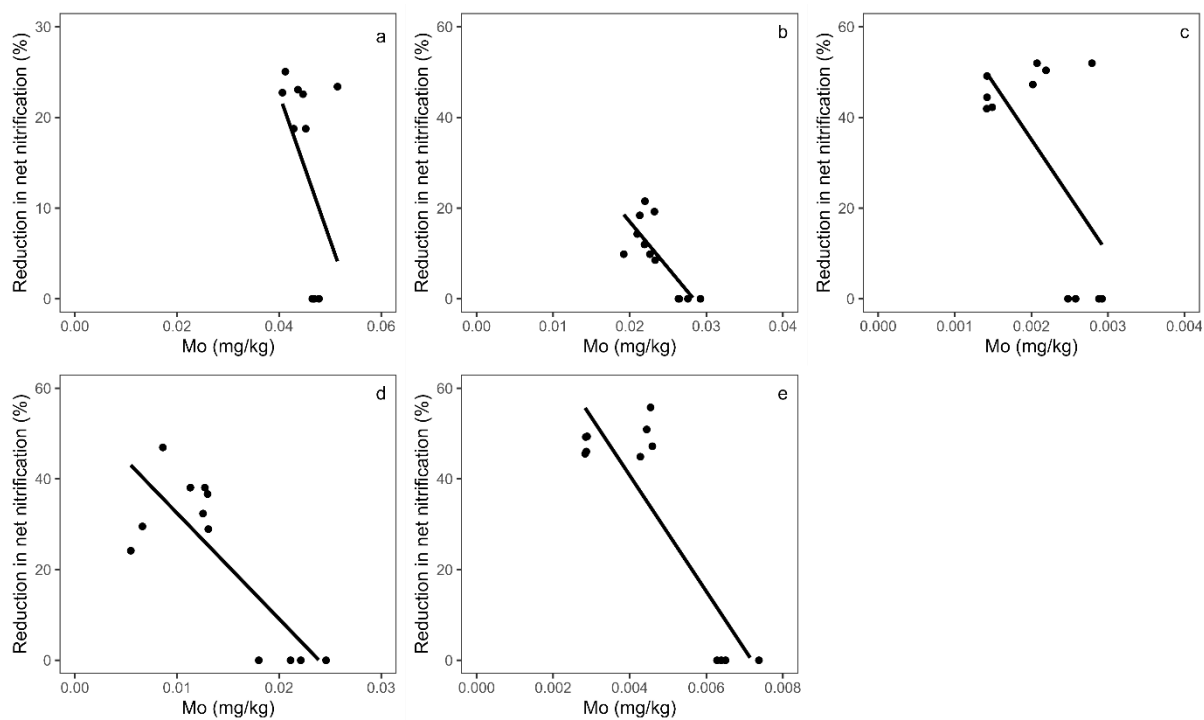
Figure 14. Relationship between Rednit and Mo in (a) 16% C soil (b) 11% C soil (c) 3% C soil (d) 1% C- CL and (e) 1% C- Sand soil at day 30 of the incubation.

3.6 Relationships between water-soluble Mo and soil properties

The results of regression analysis between water-soluble Mo and soil total C, soil total N, C:N ratio, clay+ silt content, pH, water-soluble Cu, DOC, and Fe were not consistent across all 3 levels of study. Of all these factors, water-soluble Mo was only correlated with soil total C and N content and clay + silt content globally (Fig. 15). On the basis of soil type, water-soluble Mo was strongly correlated with DOC, soil total C and N content, and C:N ratio in organic but not in mineral soils. In contrast, water-soluble Mo was a positive and significant function of pH in mineral but not in organic soils (Fig. 18, A, B). Within individual mineral soils, there were strong positive and negative correlations of water-soluble Mo with pH and DOC, respectively, but not in individual organic soils (Fig. 16 and 17). We found no correlation between water-soluble Mo and total dissolved Fe in 11% C and 16% C

soil, whereas we observed a positive and significant correlation between the two in 1%C- Sand soil. Slopes and

r^2 for all the regressions are detailed in Supplemental Table S7.
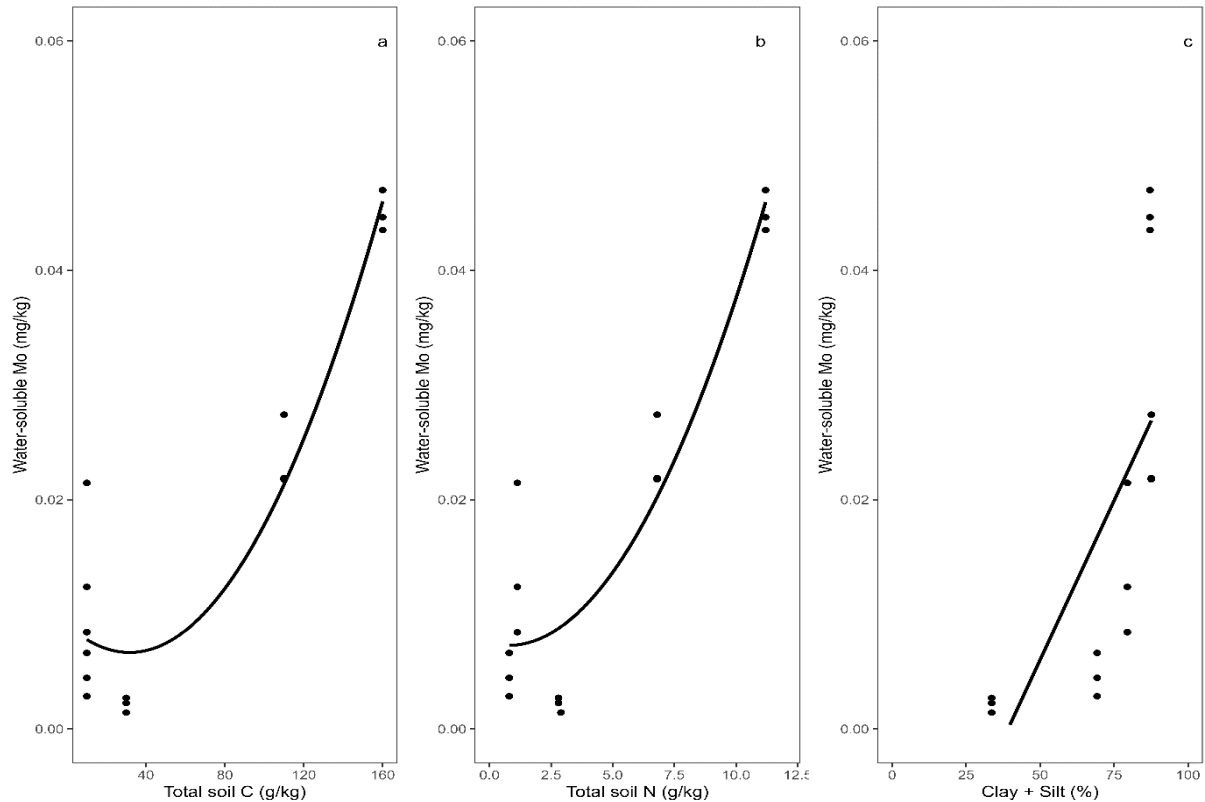
Figure 15. Relationship between water-soluble Mo, (a) soil total N (globally), (b) soil total C, and (c) clay+silt content in mineral and organic soils amended with flocs.
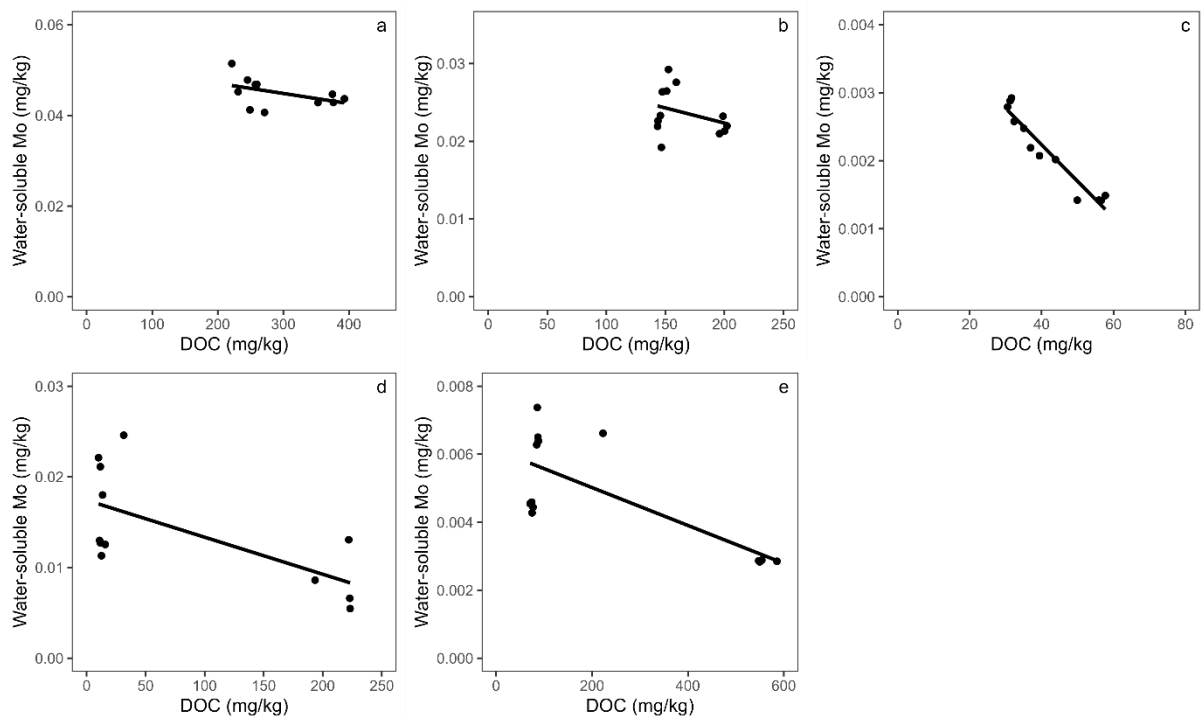
Figure 16. Relationship between water-soluble Mo and DOC in (a) 16% C soil, (b) 11% C soil, (c) 3% C soil, (d) 1% C-CL soil, (e) 1%C-Sand soil.
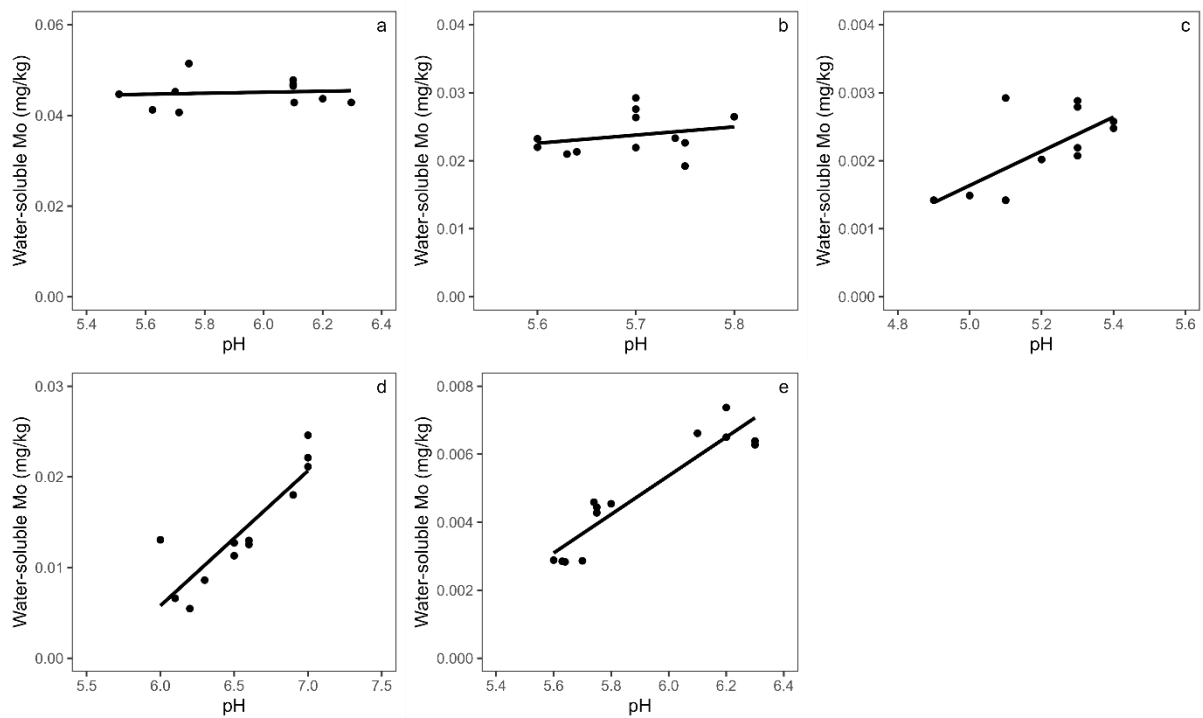
Figure 17. Relationship between water-soluble Mo and pH in (a) 16% C soil, (b) 11% C soil, (c) 3% C soi,l (d) 1% C-CL soil, (e) 1%C-Sand soil.
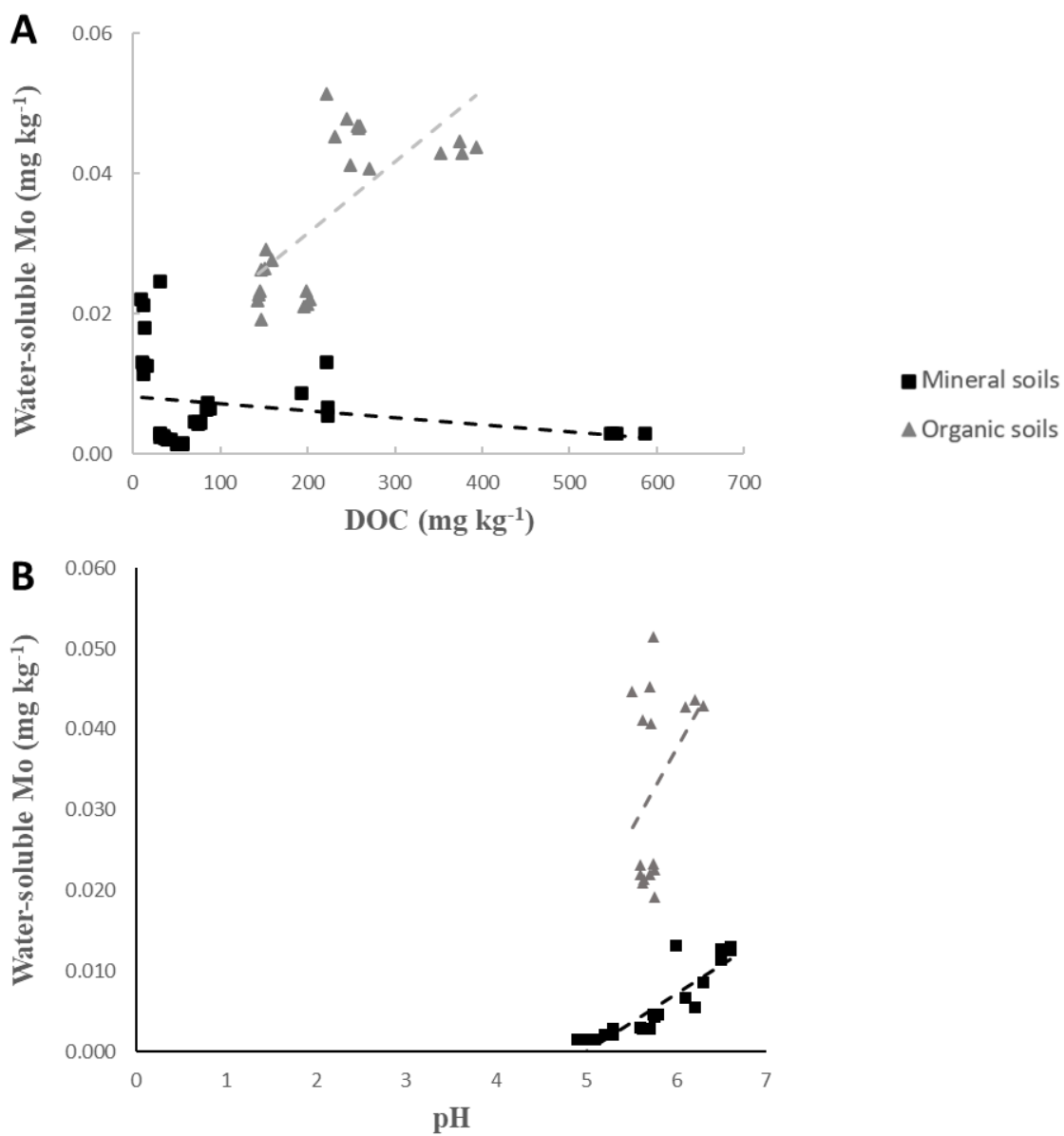
Figure 18. Relationship between water-soluble Mo, DOC (A) and pH (B) on the basis of soil category.

4 Discussion

4.1 Stability of Fe-C flocs is not equated with reactivity

The paradigm that stable flocs are not reactive originates from observations that sorption stabilizes OM against microbial degradation (Wagai and Mayer, 2007) and modifies the structural characteristics of Fe oxides, rendering them less reactive towards metals and nutrients (Henneberry et al., 2012). Our results contradict this paradigm and do not support the hypothesis that the stable aromatic floc will induce less Rednit compared to the non-stable aliphatic floc. Instead, we found that, whether stable or not, flocs reduced net nitrification similarly within each soil. This result was surprising for the following reasons:

(a) The difference in floc C chemistry is expected to affect Fe-OC floc stability. Multiple studies have reported the preferential sorption of high-molecular-weight aromatic compounds (e.g., TA) over aliphatic compounds to minerals (e.g., CMC). TA can form stable complexes with Fe(III) minerals through its phenolic groups. CMC is characterized by multiple carboxymethyl groups ($-CH_2COOH$) which also strongly chelate Fe(III). However, the strength of bonds between CMC and Fe(III) is less than that formed between TA and Fe(III) as indicated by thermal analysis.

(b) Besides thermal stability assessment, floc stability assessment in soil, as measured by the release of Fe and DOC from floc structure, showed that Fe and DOC were released in all soils amended with aliphatic but not with aromatic floc. Floc decomposition was probably due to the solubilization of Fe, a process that is mainly regulated by soil pH, mineral dissolution-precipitation dynamics, and chelation (Colombo et al., 2014):

- **In mineral soils**: pH was significantly decreased in soils amended with both floc types, but more in the aliphatic than in the aromatic floc treatments. This decrease in pH likely enabled the solubilization of both Fe and C from floc. Soluble Fe is more reactive and Fe solubility is enhanced by chelation with C

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(Colombo et al., 2014). However, Fe solubility can decrease if it precipitates as Fe mineral or coprecipitates with DOC. The occurrence of these processes might have determined the reactivity of both Fe and DOC in the soils studied in this experiment.

- **In organic soils**: pH was not significantly decreased, possibly due to the high pH buffering capacity of OM in these soils. The high content of soil C and DOC in organic soils offer opportunities for chelation and coprecipitation of Fe, which might either increase or decrease Fe solubility and reactivity, respectively.

Taken together, we posit that the aliphatic floc is more likely to release Fe and DOC in soil environment, which are more likely to subsequently engage in chemical reactions compared to Fe and OC sequestrated in the aromatic floc structure, and therefore should have resulted in more Rednit.

4.2 Rednit is related to water-soluble Mo and soil C and N content

Numerous studies have identified OM, pH, and NH_4^+ as the most important drivers of nitrification rate and extent in soil. Our study identified water-soluble Mo as a significant factor influencing nitrification in floc-amended soils. Mo bioavailability is important for N_2 fixation and NO_3^- reduction, but its potential implication in nitrification has not received much attention in soil research. However, Mo may be involved in nitrification in various ways. First, Mo is an essential micronutrient for nitrifiers (Zavarzin, 1957; Finstein and Delwiche, 1965; Tandon and Mishra, 1968). Using growth media, Finstein and Delwiche (1965) showed that the synthesis of one Nitrobacter cell requires at least 2000 atoms of Mo, and no other metal could substitute for Mo in the media. Mo also stimulated development of Nitrobacter cell mass and NO_2^- utilization but was inhibitory at greater concentrations (Finstein and Delwiche, 1965). Second, Mo is critical for the enzymatic oxidation of NO_2^- to NO_3^- performed by NXR enzyme (Zavarzin, 1958) and a deficiency in Mo halts this process (Yanase et al., 2000). This is because along with several Fe-S clusters, NXR contains a Mo center that functions as substrate (NO_2^-)-binding

site. Presumably, NO_2^- oxidation to NO_3^- occurs by a two-electron transfer from NO_2^- to Mo (VI) at the Mo center of NXR (Meincke et al., 1992).

Li et al., (2020) found soil total N is the most important determinant of nitrification globally. In fact, nitrification is highly dependent on the availability of N substrates since they stimulate the activity of the nitrifying microorganisms and their enzymes. The availability of substrates stimulates N mineralization as well which may increase soil nitrification (Li et al., 2020). However, another study by Sahrawat, (1982) found that organic C and total N content of soils were not related to nitrification, but more to soil pH, across a set of 8 tropical soils. While we cannot make direct comparisons of how soil factors controlled Rednit in our study and nitrification in the above-mentioned studies, due to the fact that nitrification was not normalized to a control soil in the latter, we found that in comparison to mineral soils, net nitrification was high in organic soils characterized by high C and N content which is similar to the trend observed by Li at al., (2020), but not to Sahrawat, (1982). Although NH_4^+ concentrations decreased in all soils amended with floc (data not shown), this decrease was not significant within each soil type (Supplemental Table S7). This suggests that nitrification was not substrate limited. While it is possible that other mechanisms contributed to reduced nitrification in soils amended with flocs, we posit that increased soil C and N affected Mo availability, which in turn reduced nitrification. While it is possible that other mechanisms have contributed to reduced nitrification in soils amended with flocs, we posit that increased soil C and N might have affected Mo availability which in turn reduced nitrification in our soils.

4.3 Mo bioavailability is limited in soils amended with flocs

Mo bioavailability is controlled by sorption and desorption dynamics in soil. In our experiment, soil C content, DOC, pH, and Fe appeared to   exert control on Mo bioavailability.

4.3.1 Relationship between water-soluble Mo, DOC, and total soil C

In agreement with our results, previous work has reported a positive and significant relationship between soil C content and readily exchangeable/available Mo (Lombin, 1985; Marks et al., 2015; Rutkowska et al., 2017), indicating that water-soluble Mo originates from SOM mineralization and that high levels of water-soluble Mo are generally associated with a high content of OM. This is in accordance with the observation that organic soils had more water-soluble Mo than mineral soils at day 30 of the incubation experiment. In addition to serving as a reservoir of Mo, SOM mediates its retention, storage, and bioavailability (Karimian and Cox, 1978; Wichard et al., 2009; Marks et al., 2015). First, Wichard et al., (2009) found that Mo adsorption prevents Mo leaching and promotes its acquisition by N_2 fixers in topsoil. These microorganisms employ small complexing agents called metallophores that capture Mo from SOM-Mo complexes prior to incorporation into the nitrogenase enzyme. Second, SOM can also promote Mo bioavailability by inducing the micro-aggregation of iron oxides, which results in decreased penetration of MoO_4^{2-} into iron oxide micropores (Lang and Kaupenjohann, 2003). This mechanism was proposed following the observation that higher MoO_4^{2-} desorption occurred in soil samples with high C concentrations compared to desorption from low-C subsoil (Lang and Kaupenjohann, 2003). Third, SOM adsorption can increase the bioavailability of Mo to plants by restricting Mo interactions with Fe, Mn, and Al oxides (Jenne, 1977). Besides high rates of Mo mineralization from SOM, these processes have the potential to slow down MoO_4^{2-} immobilization on floc or its decomposition products in organic soils, which may explain why water-soluble Mo was not reduced as dramatically as in mineral soils.

In contrast to organic soils, DOC appeared to have a negative effect on Mo bioavailability in mineral soils. This observation agreed with the study of Gupta, (1971) who found that the addition of organic materials decreased exchangeable soil Mo, indicating that Mo was fixed with increased OM content of soil. Accordingly, Xu et al., (2013) found that the concentration of Mo in soil solution decreased with increasing soil OC. It is possible that in

mineral soils, DOC restricted Mo bioavailability by the establishment of strong binding mechanisms, such as ligand exchange and specific adsorption processes (Xu et al., 2013). Lombin, (1985) also postulated that Fe bound in DOM can be responsible for Mo adsorption. Moreover, it is possible that Mo was   incorporated into floc structure. For instance, molybdate ion can be incorporated into hydrotalcite minerals that are formed by the precipitation of Fe or Al oxides (Allada et al., 2002; Smith et al., 2005; Paikaray and Hendry, 2013). Natural Mo mineralization in these soils is probably low due to low C content and fails to compete with the negative effects of added C on Mo bioavailability.

4.3.2 Relationship between water-soluble Mo and clay+ silt and sand contents

In agreement with our study, it has been found that available Mo content increases with increased fineness of soil texture (assessed by clay+ silt content) (Srivastiva and Gupta, 1996). In fact, Mo is readily adsorbed by kaolinite, illite, and montmorillonite (Goldberg et al., 1996), and a high percentage of clay generally corresponds to a higher percentage of available Mo (Lombin, 1985). However, soils with the highest clay content had the lowest Mo bioavailability in our study. This is probably due to the nature of the charges on clay which depend on pH and point of zero charges (PZC). Indeed, positively charged clays are more likely to adsorb MoO_4^{2-} and lower its bioavailability than negatively charged clays.

Sandy soils are generally devoid of nutrients, which may explain in part why 1% C Sand had low Mo bioavailability. In addition, Bloomfield and Kelso (1973), Karmian and Cox (1978) and Riley et al., (1987) found that Mo is susceptible to leaching in sandy soils, depending on soil pH.

4.3.3 Relationship between water-soluble Mo, pH, and Fe

Soil pH has been cited as an important control on Mo bioavailability. It is known that Mo bioavailability is limited in soils with pH less than 5.5 due to adsorption with soil colloids such as Fe oxides (Duval et al., 2015). Lindsay

et al., (1972) found that the concentration of MoO_4^{2-} increases 100-fold for each unit increase above pH 3, due to lower activity of Fe and Al minerals, an increase in free negatively charged soil colloids, and stronger competition between molybdate and hydroxyl anions for adsorption sites (Jarrell and Dawson, 1978; Jiang et al., 2015). Most of our floc-amended mineral and organic soils had an average pH of 5.5 at day 30 of the incubation, suggesting possible adsorption of Mo on Fe species. Adsorption is likely in the case of mineral soils; for instance, we found that in 1% C- Sand soil, the decline in water-soluble Mo concentrations (from 0.0066 to 0.0029 mg.kg^{-1} soil) was accompanied by a decrease in pH (from 6.2 to 5.6) and increased soluble Fe (from 0.24 to 6.16 mg.kg^{-1}). Water-soluble Mo and Fe were positively and significantly correlated ($r^2= 0.94***$) suggesting that Fe species adsorb Mo in this soil. In contrast, Marks et al., (2015) found that SOM was more important for Mo complexation than short-range Fe, Mn, and Al oxides, given that 33% of bulk soil Mo was associated with SOM, compared to 1.4% associated with these minerals, in soils spanning a wide range of pH (6.45–4.55). In contrast to our mineral soils, water-soluble Mo was not correlated with pH in organic soils although they had similar pH. This result agrees with Perakis and Sinkhorn, (2011) and Marks et al., (2015) in that pH restricts the availability of Mo only in soils with lower OM content and relatively low soil C.

4.4 Connecting the dots: floc-induced Mo depletion affects nitrification in soils

This study combines floc-mediated Mo availability and nitrification into one environmental model (Fig. 1). The results presented here indicate that floc or its decomposition products (Fe and DOC) are effective at removing Mo from soil solution, thereby reducing nitrification in mineral soils. We posit that Mo removal occurs as a result of either incorporation into the stable floc structure in the case of the aromatic floc, or sorption onto DOC and/or Fe in the aliphatic floc treatment; both processes are promoted by decreased pH caused by the presence of floc. We also posit that nitrification is halted at the final step: NO_2^- oxidation to NO_3^-. This was confirmed by the fact that NO_2^- concentrations increased significantly in 1% C Sand and 1% C Clay (except in the aromatic floc treatment),

suggesting that NO_2^- accumulated in these soils (Fig.8). Although similar trend was observed in 11% C soil amended with flocs, the accumulation of NO_2^- was not significant. Overall, this mechanism appears to be active in soils with less than 3% C content.

In organic soils, net nitrification was not affected by floc-mediated Mo reduction due to their high OM content. Indeed, OM may increase Mo availability in several ways: (a) by enhancing Mo chelation, (b) by enhancing the supply of Mo by SOM mineralization, and/or (c) by occupying adsorption sites on flocs and/or causing the micro aggregation of Fe oxides, thereby limiting the capacity of flocs to adsorb Mo. As a result, nitrification was not inhibited and NO_2^- did not accumulate, as demonstrated for the 11% C- soil.
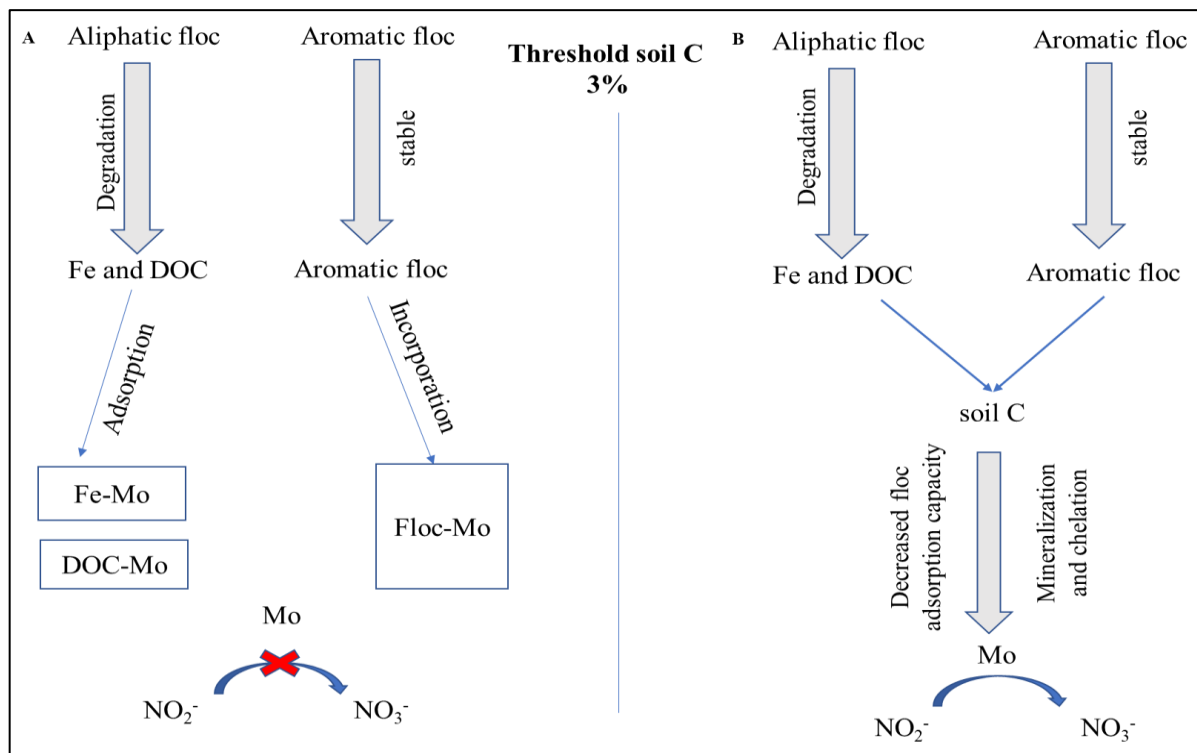
Figure 19. Mechanisms of floc-mediated Rednit in mineral soils (A) and organic soils (B).

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4.5 Response of nitrification to Mo addition in 1%C- soil amended with flocs

Results showed that none of our supplemental Mo additions improved net nitrification (Fig. 20) compared to the control treatments. Moreover, water-soluble Mo increased with Mo addition only after the addition of 100 mg.kg^{-1} (Fig. 21). This suggests that below this concentration, Mo adsorption by floc or its decomposition products was still operative. There was also no correlation between water-soluble Mo and NO_3^- concentrations in all Mo addition treatments (Data not shown).

These results can have three possible explanations:

(a) The amount of Mo that saturates the adsorption capacity of floc or its decomposition products and still leaves enough Mo for microbial utilization was not reached even at the maximum amount of supplemental Mo. Indeed, nitrification is expected to resume if enough of Mo is applied, as demonstrated in Wen et al., (2019). These authors found that the application of 0.3 mg.kg^{-1} Mo increased NO_3^- concentrations in an acidic soil (pH=4.99).

(b) Added Mo was toxic to nitrifiers; Liang et al., (1978) found that the application of 480 mg Mo/kg inhibited nitrification by 39 to 74% and caused the accumulation of NO_2^- in three different soils. However, we discard this possibility in our soils because net nitrification in floc-amended soil was similar with and without added Mo (Fig. 20), indicating that decreased net nitrification originates as a result of floc addition and not from added Mo. Additionally, Ueda et al., (1988) demonstrated that an addition of Mo up to 1000 mg.kg^{-1} as sodium molybdate was not toxic, while Buekers et al., (2010) determined that the toxicity threshold is 3129 (2910-3363) mg.kg^{-1} expressed as total Mo in soil.

(c) Floc, its decomposition products, or added Mo causes an imbalance in the concentrations of one or more other nutrient/metals required for growth of nitrifiers or the nitrification process. For instance, the growth

of a denitrifying microorganism in a medium that was initially depleted in Mo by Fe and Al precipitation

was not fully restored by the addition of Mo, because of possible lack of other nutrients (Ge et al., 2018).
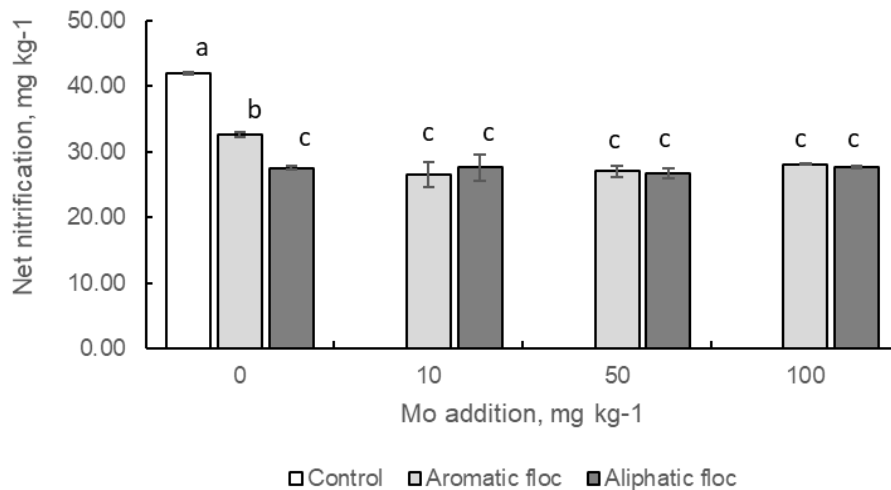
Figure 20. Net nitrification in soils amended with aliphatic and aromatic flocs and increased Mo addition at day 30 of the incubation. Values are average of 4 repetitions per treatment.
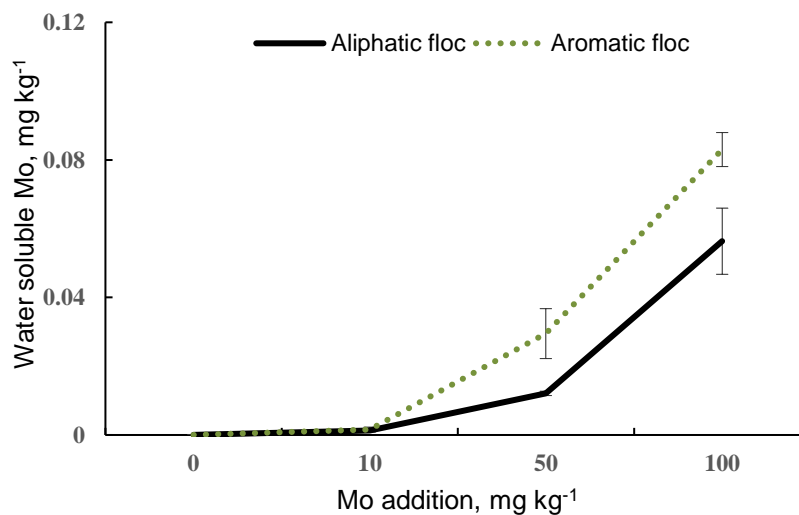
Figure 21. Water-soluble Mo in relationship with added Mo at day 30 of the incubation. Values are average of 4 repetitions per treatment.

5. Implication for the environment and management

Flocs in soil can originate from natural coprecipitation of metals and DOM. Floc formation is routinely used in engineered systems like wastewater treatment or on-site agricultural drainage treatment facilities, which use iron-based coagulants to remove DOM from the waters or constructed wetlands. Flocs are shown to reverse land subsidence and enhance C sequestration due to their high stability in wetland environments. However, the present study raises questions on the importance of stability of flocs for soil C and N sequestration. It is often assumed that stable flocs are not reactive, based on the observation that C bioavailability is constrained, resulting in slower C cycling. Our study shows that stable structures can be reactive towards other nutrients (like Mo), which may constrain C and N sequestration. Indeed, Van Groenigen et al., (2006) found that soil C sequestration is restricted by N availability and nutrients that support N_2 fixation, such as Mo, phosphorus, and potassium. Therefore, adopting a more holistic approach that considers the effect of flocs on other soil nutrients can bring new insight into mechanisms of C and N sequestration and offer new possibilities for the use of flocs in management. Soil properties and conditions should also be considered in this holistic approach. In fact, our study showed that nitrification in organic soils appeared to be less sensitive to the application of flocs compared to mineral soils.

This study also demonstrates that the application of flocs decreases soil nitrification by inducing Mo deficiency in mineral soils. This decrease in net nitrification was accompanied by a minimal decrease in NH_4^+. Potentially, flocs can be used as nitrification inhibitors in agricultural systems and increase the availability of N to plants, for example as a coating on fertilizer preparations. Decreased nitrification from agricultural fields can alleviate other environmental issues such as eutrophication of lakes and water pollution. Mo deficiency can also limit denitrification and N_2 fixation by decreasing the activity of NO_3^- reductase and nitrogenase enzymes, respectively.

Supplemental Tables

Supplemental Table S6: Factor loadings for the first five principal components (PC1–PC5)

	PC1	PC2	PC3	PC4	PC5
Rednit	0.18	0.06	0.47	0.35	0.72
DOC	-0.26	-0.21	0.45	0.19	-0.27
Fe	-0.26	-0.17	0.42	0.34	-0.38
Mo	-0.43	0.007	-0.18	0.13	0.10
Cu	-0.04	0.55	0.12	0.26	-0.01
pH	-0.03	-0.42	-0.40	0.43	-0.001
C	-0.44	0.17	-0.04	-0.08	0.12
N	-0.43	0.20	-0.04	-0.02	0.08
CN	-0.36	-0.04	0.21	-0.54	0.15
Sand	0.12	-0.48	0.29	-0.33	0.11
Clay + silt	-0.31	-0.34	-0.20	0.13	0.42

Supplemental Table S7: Results of two-way ANOVA on the effects of days treatment and days on net NH4+ production over the course of the incubation

Soil type	Main effect of treatment	Main effect of days	Interaction effect
16% C soil	F= 0.676 p=0.512	F= 53.953 p= 1.72e-10 ***	F=0.407 p=0.667
11% C soil	0.346 0.708	F=74.730 p=5.28e-13 ***	0.099 0.906
3% C soil	6.163 0.00327 **	182.625 p< 2e-16 ***	1.962 0.14738
1% C- Clay	0.572 0.567	101.417 1.06e-15 ***	1.353 0.265
1% C- Sand	1.825 0.168	34.935 8.5e-08 ***	0.935 0.397

Supplemental Table S8: Regression analysis results for relationships between Rednit and soil properties

Variable	Level of study	Slope	Intercept	Adjusted r2	P value
	Global	-0.52	70.64	0.52	9.26E-08***
	Soil category				
Clay+ silt fraction	Organic soils	-16.32	1444.68	0.47	0.0019***
	Mineral soils	-0.20	55.74	0.20	0.014618
	Global	-0.0008	0.042	0.52	7.73E-08***
	Soil category				
	Organic soils	0.0015	0.0054	0.46	0.002319**
	Mineral soils	-0.0003	0.018	0.36	0.0013***
	Individual soils				
Water-soluble Mo	1%C-Sand	8.95E-05	-0.00069	-0.0063	0.36 NS
	1%C-Clay	0.00012	0.0060	-0.064	0.47 NS
	3% C	9.32E-05	-0.002	0.51	0.027**
	11% C	3.01E-05	0.021	-0.15	0.79 NS
	16% C	7.32E-05	0.042	-0.16	0.89 NS
	Global	-0.007	6.005	0.045	0.099 NS
	Soil category				
	Organic soils	0.0019	5.73	-0.069	0.86 NS
	Mineral soils	-0.036	7.29	0.28	0.0039***
pH	**Individual soils**				
	1%C-Sand	0.007	5.34	-0.0083	0.37 NS
	1%C-Clay	0.014	5.84	0.069	0.26 NS
	3% C	0.021	4.12	0.23	0.12 NS
	11% C	-0.012	5.85	0.86	0.0005***

	16% C	-0.057	7.10	0.11	0.22 NS
Soil total C	Global	-3.25	172.21	0.58	5.09E-09***
	Soil category				
	Organic soils	3.40	73.98	0.47	0.002***
	Mineral soils	0.40	-0.91	0.079	0.097 NS
Soil total N	Global	-0.02	1.12	0.52	9.36E-08***
	Soil category				
	Organic soils	0.03	0.36	0.47	0.002***
	Mineral soils	0.001	0.093	-0.022	0.49 NS
C:N ratio	Global	-0.073	14.62	0.19	0.0029***
	Soil category				
	Organic soils	0.053	13.38	0.47	0.002***
	Mineral soils	0.16	3.57	0.46	0.00014***
DOC	Global	-1.61	245.29	-0.0053	0.37 NS
	Soil category				
	Organic soils	10.69	48.64	0.38	0.0061***
	Mineral soils	1.22	105.82	-0.042	0.80 NS
	Individual soils				
	1%C-Sand	-22.69	1420.30	-0.052	0.45 NS
	1%C-Clay	-5.74	311.45	-0.0028	0.36 NS
	3% C	-2.25	153.48	0.79	0.0017***
	11% C	5.36	95.83	0.80	0.0015***
	16% C	-7.16	463.82	-0.095	0.55 NS
Fe	Global	-0.010	2.26	-0.020	0.65 NS
	Soil category				
	Organic soils	0.22	-1.41	0.28	0.019

	Mineral soils	0.059	-1.16	0.008	0.28 NS
	Individual soils				
	1%C-Sand	-0.20	13.50	-0.09	0.55 NS
	1%C-Clay	0.017	-0.009	0.35	0.07 NS
	3% C	0.003	0.18	-0.15	0.83 NS
	11% C	0.032	0.40	0.29	0.09 NS
	16% C	-0.25	9.76	0.021	0.32 NS

Supplemental Table S9: Relationships between water-soluble Mo and soil properties

Variable	Level of study	Slope	Intercept	Adjusted r^2	P value
	Global	2974.13	142.93	0.05	0.07 NS
	Soil category				
	Organic soils	5601.81	55.78	0.52	0.000935***
	Mineral soils	-10017.82	212.33	0.0026	0.31414 NS
	Individual soils				
DOC	1%C-Sand	-298986	1411.98	0.98	6.36E-07***
	1%C-Clay	-24260.8	366.93	0.48	0.05*
	3% C	-19385.3	82.28	0.89	0.00025***
	11% C	709.33	156.58	-0.16	0.93 NS
	16% C	-7156.49	624.1580964	-0.037	0.42 NS
	Global	3599.34	5.08	0.86	3.16E-18***
Soil total C	**Soil category**				
	Organic soils	2148.03	64.21	0.95	7.02E-11***
	Mineral soils	-1449.65	24.81	0.36	0.0012***

Clay+silt fraction	Global	858.06	57.38	0.44	2.18E-06***
	Soil category				
	Organic soils	-19.55	88.02	0.95	7.02E-11***
	Mineral soils	3512.23	42.14	0.53	5.25E-05***
Sand content	Global	-768.029	41.13	0.12	0.016***
	Soil category				
	Organic soils	19.55	11.97	0.95	7.02E-11***
	Mineral soils	-1303.93	46.12	-0.024	0.509945*
Clay content	Global	-137.02	28.16	0.000667	0.31 NS
	Soil category				
	Organic soils	-206.22	28.56	0.95	7.02E-11***
	Mineral soils	2298.32	16.47	0.29	0.0034***
pH	Global	7.12	5.63	0.045	0.099 NS
	Soil category				
	Organic soils	7.42	5.52	0.087	0.14
	Mineral soils	112.81	5.13	0.79	3.49E-09***
	Individual soils				
	1%C-Sand	72.82	5.43	0.75	0.0033***
	1%C-Clay	37.78	5.95	0.49	0.20
	3% C	248.43	4.69	0.62	0.011**
	11% C	-7.64	5.84	-0.13	0.71 NS
	16% C	-12.93	6.43	-0.14	0.72
	16% C	154.46	0.40	0.82	0.0010***
C:N ratio	Global	93.97	10.62	0.38	1.1E-05***
	Soil category				
	Organic soils	33.93	13.22	0.95	7.02E-11***

	Mineral soils	-282.34	12.21	0.34	0.0015***
Soil N content	Global	23.62	0.072	0.87	3.52E-19***
	Soil category				
	Organic soils	19.05	0.27	0.95	7.02E-11***
	Mineral soils	-9.13	0.21	0.13	0.043*
	Global	56.54	0.97	0.14	0.0088**
	Soil category				
	Organic soils	141.56	-2.07	0.60	0.00023***
	Mineral soils				
Fe	**Individual soils**				
	1%C-Sand	-110.08	2.02	0.00088	0.32 NS
	1%C-Clay	No observed released of Fe			
	3% C	No observed released of Fe			
	11% C				
	16% C	-189.92	12.69	0.026	0.31 NS

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CHAPTER III: A conceptual framework of the effect of iron-organic carbon coprecipitates on nitrification in soils

Abstract. Although iron (Fe)-organic carbon (C) coprecipitates (or flocs) are important soil components affecting C cycling, their effects on nitrogen (N) cycling processes, especially nitrification, are not well known. Nitrification, an important pathway of N transformation in soils, converts ammonium (NH_4^+) to nitrate (NO_3^-) using metalloenzymes, the activity of which depends on iron (Fe), molybdenum (Mo), and copper (Cu) availability. Besides Fe, Mo and Cu, the growth of the nitrifying communities depends on other soil nutrients such as phosphorus (P), but excess nutrients can induce toxicity and jeopardize microbial processes. Here, we used flocs of different chemistry (aromatic and aliphatic) and known Fe and C composition to investigate their effects on soil nutrient status and link to nitrification in soils along a soil C gradient. A previous study (Chapter II) found that both aromatic and aliphatic flocs reduced net nitrification, but the magnitude of their effect was more pronounced in mineral than in organic soils. In the present study, we found that flocs increased water-soluble P, Fe, Cu, P, nickel (Ni), zinc (Zn), manganese (Mn), magnesium (Mg), aluminium (Al), cobalt (Co), calcium (Ca) and potassium (K), but decreased water-soluble Mo in mineral soils. However, flocs did not affect nutrient concentrations in organic soils. The enhanced solubility of nutrients in mineral soils was possibly due to increased availability of chelating agents, whereas decreased Mo bioavailability was due to its incorporation into or adsorption to flocs or their decomposition products. In organic soils, organic matter (OM) likely limited floc adsorption capacity of Mo and/or immobilized nutrients. The link between floc-induced changes in soil nutrient status and nitrification is discussed in light of current knowledge on the influence of nutrients on microbial processes. We apply the principle of Liebig's Law of the Minimum and knowledge on nutrient toxicity to microbial communities to develop a conceptual framework of the possible effects of flocs on nitrification in agricultural soils.

1 Introduction

Nitrification is a fundamental process in the nitrogen (N) cycle, which converts NH_4^+ to NO_3^- using metalloenzymes such as ammonia monooxygenase (AMO) and nitrite oxidoreductase (NXR). The former enzyme catalyses the conversion of ammonia (NH_3) to hydroxylamine (NH_2OH) whereas the latter enzyme converts nitrite (NO_2^-) to NO_3^- (Lancaster et al., 2018). To ensure these reactions, AMO and NXR use metal cofactors, namely copper (Cu) and molybdenum (Mo) (respectively). The availability of these cofactors in soils can be controlled by interactions with iron (Fe)-organic carbon (C) coprecipitates as influenced by soil properties (Chapter II).

Iron (Fe) oxides, hydroxides and oxyhydroxides (collectively referred to as iron oxides (Fe-oxides)) often coprecipitate with dissolved organic matter (DOM) in soils and sediments that experience significant changes in pH and redox conditions. The resulting coprecipitates, which are commonly called flocs, are critical to soil nutrient cycling since they promote the long-term storage and sequestration of carbon (C) (Kaiser and Guggenberger, 2000; Lalonde et al., 2012; Lehmann and Kleber, 2015) and influence metal mobility and bioavailability (Henneberry et al., 2012). In wastewater treatment, the removal of DOM via coagulation by Fe-based salts is a common practice that resembles the natural coprecipitation of Fe and DOM. This technique intends to remove organic particles from polluted waters and improve water quality (Hansen et al., 2018). The resulting flocs are generally removed from the water column and disposed of in landfills. However, many studies have shown that retaining flocs in constructed wetlands has benefits such as addressing land subsidence by building land elevation and promoting C accumulation and sequestration (Bachand et al., 2000; Bachand et al., 2006; Downing et al., 2008; Henneberry et al., 2012; Stumpner et al., 2018).

In addition to their documented effects on soil C cycling, we showed that model flocs, made by reacting iron sulfate with aromatic and aliphatic C compounds, reduce and/or inhibit nitrification by restricting Mo bioavailability in soils (Chapter II). However, supplying additional Mo did not restore nitrification in a soil amended with flocs, leading to the hypothesis that the presence of flocs affected one or more other nutrients

required for nitrification or the growth of nitrifiers. For instance, Fe can limit the bioavailability of phosphorus (P) (Qualls et al., 2009), which is an essential nutrient for nearly all microbes. Currently, there is limited information on the possible influence of Fe-based flocs on other important soil nutrients, such as P, nickel (Ni), zinc (Zn), manganese (Mn), magnesium (Mg), aluminium (Al), cobalt (Co), calcium (Ca) and potassium (K). The availability of these elements can be controlled by interactions with OM and the highly sorptive Fe minerals, which may influence nitrification. While many are essential nutrients, the nitrifying communities are especially sensitive to excess nutrients and a toxicity response maybe be also triggered. However, nutrient thresholds specific for the nitrifying communities in soils are not generally determined.

In this study, we test the hypothesis that besides Mo, flocs reduce the availability of other nutrients required for nitrification in soils. To do so, we use the same floc compounds from our previous study (Chapter II) to investigate their effects on the availability of P, Al, Ni, Zn, Mn, Mg, Cu, Co, Ca, and K in soils with different properties (e.g., C, N, and clay content). These compounds are characterized by different chemistry (aromatic and aliphatic) and known Fe and C composition. We discuss how the results of this study fit into the current knowledge on the influence of nutrient on microbial processes and propose a more generalized conceptual framework of the effects of Fe-OC flocs on soil nitrification. We also propose ways to move the knowledge forward on the possible use of flocs in agriculture.

2 Materials and methods

2.1 Preparation of Fe-C flocs and soils

All the chemicals were purchased from Sigma-Aldrich. Tannic acid (TA) and carboxymethyl cellulose (CMC) were used to prepare the aromatic and aliphatic flocs, respectively. To prepare the aromatic floc, 10 mL of a 2 % (v/v) TA solution and 18 mL of 2 % (v/v) iron (III) sulfate (FeS) solution were prepared using deionized water

(DI). In an Erlenmeyer flask, FeS solution was added to TA solution under vigorous stirring, and the pH was adjusted to 7 by 1 M NaOH. The resulting black solution was centrifuged at 10000 rpm for 15 min, after which the supernatant was discarded, and the precipitate (floc) was washed 3 times with DI water to remove excess Fe. Afterwards, floc was oven-dried overnight at 30^0C and ground using a pestle and mortar to obtain a fine powder. Powdered floc was stored at room temperature in a closed container. To prepare the aliphatic floc, 3 mL of 2 % (v/v) FeS solution was added to 10 mL of a 2 % (v/v) CMC solution in falcon tubes. The resulting solution was shaken manually and centrifuged at 10000 rpm for 15 min, after which the supernatant was discarded, and the precipitate (floc) was washed 3 times with DI water to remove excess Fe. The floc was freeze-dried and ground using a pestle and mortar to obtain a fine powder before storing it at room temperature in a closed container.

2.1.1 Characterization of Fe-C flocs composition

Elemental analyses of total C and N were performed on powdered flocs using an elemental analyzer (EAS 4010, Costech Analytical Technologies Inc., Valencia, CA). Floc Fe content was determined by digesting 30 mg of floc in 1mL nitric acid HNO_3^- (69 %, 16 M) followed by ferrozine-based colorimetric assay. The results of these analyses are presented in Table 10. Besides these analyses, we measured water extractable nutrients from flocs. To do so, hot water extraction was performed on 40 and 36 mg of the aromatic and the aliphatic floc (respectively) which received 20 mL of DI water. Samples were put in a hot water bath for 1h with occasional shaking. Afterwards, samples were shaken for 1h, centrifuged (10.000 g for 10 min) and passed through 0.45 μm syringe filter to remove organic material. Dilution with 3% nitric acid (HNO_3) followed, after which samples were analyzed by Inductively Coupled Plasma (ICP) at the ICP laboratory at the University of California at Davis. Results are presented in Table 11.

Table 10: C, N and Fe content of floc (means ± standard error, n = 3)

Floc	C (mg.kg^{-1})	N (mg.kg^{-1})	Fe (mg.g^{-1})
Aromatic floc	22.5 ± 0.37	ND*	1.4 ±0.31
Aliphatic floc	27.3 ± 0.08	ND*	0.26 ±0.04

*ND: Not detectable

Table 11: Water-extractable nutrients from the aromatic and the aliphatic flocs (means ± standard error, n = 3)

	Ni (mg/l)	Al (mg/l)	K (mg/l)	Ca (mg/l)	Mn (mg/l)	Co (mg/l)	Zn (mg/l)	Mg (mg/l)	Cu (mg/l)	P (mg/l)	Mo (mg/l)	Fe (mg/l)
Aromatic floc	0.05± 0.002	1.42± 0.36	16.5± 8.46	0.40± 0.07	0.51± 0.018	0.004±0 .0001	0.009 ±0.0003	0.023 ±0.001	0.0035± 0.0002	0.042± 0.001	0.0001± 5.2E-6	5.17± 0.54
Aliphatic floc	0.019± 0.0009	0.03± 0.006	20.3± 17.2	0.87± 0.13	0.28± 0.020	0.002±0 .0002	0.021± 0.056	0.08± 0.045	0.0032± 0.001	0.024± 0.001	0.0002± 5.56 e-6	115.85± 0.43

2.1.2 Soil preparation

Soils along a soil C gradient (1%, 3%, 11% and 16 %) were collected from the top 0-15 cm from Twitchell Island, located on the western portion of the San Francisco Bay Delta (California, USA). An additional 1% C soil was collected from Russel Ranch Sustainable Agricultural Facility (RRSAF) at the University of California at Davis. Most soil from Twitchell island is Rindge mucky silt loam (Euic, thermic Typic Haplosaprist), formed from Tule and reed deposition. The Twitchell Island 1% C soil has a sandy texture, whereas RRSAF soil is a clay texture. For each soil, five samples were randomly collected, composited, and sieved (2mm). Visible plant residues and roots were removed and soils were air-dried and stored until use. Gravimetric water content (GWC) (105°C for 48–120 h) and Water Holding Capacity (WHC) of soils were determined. The percentages of clay, silt and sand were determined by hydrometer method, with a pre-treatment with H_2O_2 to remove OM. pH was determined in 1:5 soil to water slurries. Selected soil properties and initial metal concentrations are presented in Tables 12 and 13, respectively.

Table 12: Selected properties of soils (means ± standard error, n = 4 where indicated)

Soil	C (%)	N (%)	C:N ratio	Sand (%)	Clay (%)	Silt (%)	pH
16% C	16 ± 0.20	1.12 ± 0.01	14.73 ± 0.08	12.8	19.4	67.8	6.1 ± 0.00
11% C	11 ± 0.28	0.68 ± 0.00	13.94 ± 0.17	12.4	24.2	63.4	5.9 ± 0.02
3% C	3 ± 0.06	0.28 ± 0.03	10.7 ± 0.05	5.4	32.4	62.3	6.6 ± 0.07
1% C- CL	1 ± 0.03	0.13 ± 0.02	8.53 ± 0.01	20.5	46.7	32.8	7.8 ± 0.02
1% C- Sand	1 ± 0.04	0.08 ± 0.00	13.13 ± 0.43	91.7	7.0	1.3	6.5 ± 0.02

Table 13: Initial water-soluble metal and DOC concentrations in soils (means ± standard error, n = 3)

Soil	Ni (mg.kg⁻¹)	soluble Al (mg.kg⁻¹)	soluble K (mg.kg⁻¹)	Water soluble Ca (mg.kg⁻¹)	Water soluble Mn (mg.kg⁻¹)	Water soluble Co (mg.kg⁻¹)	Water soluble Zn (mg.kg⁻¹)	Water soluble Mg (mg.kg⁻¹)	Water soluble Cu (mg.kg⁻¹)	Water soluble P (mg.kg⁻¹)	Water soluble Mo (mg.kg⁻¹)	Water soluble Fe (mg.kg⁻¹)	DOC (mg.kg⁻¹)
16% C	0.12± 0.06	2.86± 0.12	16.27± 0.52	119.75 ±0.64	0.98± 0.006	0.014± 0.0005	0.009± 0.0003	69.73± 1.44	0.053± 0.003	2.81± 0.05	0.025± 0.001	4.38 ± 0.42	837.43 ± 3.27
11% C	0.072± 0.001	2.06± 0.06	21.2± 0.54	89.99± 0.84	1.92± 0.01	0.018± 0.0002	0.011± 0.002	47.91± 1.54	0.042± 0.0006	1.54± 0.03	0.017± 0.0001	1.29± 0.05	391.21 ± 2.47
3% C	0.1±0.06	0.07± 0.04	4.29± 3.52	40.02± 19.91	0.47± 0.05	0.004± 0.002	0.003± 0.001	25.71± 6.57	0.048± 0.014	1.91± 2.89	0.009± 0.01	0.16± 0.00	139.26 ± 0.85
1% C CL	0.13±0.06	0.17± 0.11	6.33± 3.43	33.33± 19.98	0.42± 0.07	0.005± 0.002	0.0033 ±0.0003	24.10± 6.21	0.052± 0.01	3.55± 2.87	0.014± 0.009	0.61± 0.00	73.09± 4.97
1% C Sand	0.055 ±0.002	1.13± 0.32	20.11± 0.63	30.30± 0.32	1.04± 0.02	0.009± 0.0005	0.008± 0.002	16.06± 0.73	0.027± 0.002	0.56± 0.03	0.0031± 8.49e-05	3.42± 0.02	239.6± 0.79

2.2 Incubations

Flocs were applied to 6g air-dried soil at a rate providing 2mg C/g soil. Flocs were thoroughly mixed with soils and incubated at 65 % of the WHC under 30°C for 30 days. Control samples were prepared by incubating soil without flocs. Each treatment was replicated four times. The soil-floc mixture was placed in a specimen cup, which was placed in a mason jar containing 2 ml of DI water to maintain moisture. The jars were closed with a foam lid to allow gas exchange. NH_4^+, NO_3^-, dissolved organic C (DOC) were analyzed on days 0, 3, 5, 7, 10, 17 and 30 of the incubation. Net N mineralization and nitrification ($mg\,N\,kg^{-1}$ dry soil) in each treatment were calculated as the difference between the final and initial NH_4^+ and NO_3^--N contents, respectively. Reduction in net nitrification (Rednit) was calculated as the percent difference between NO_3^--N contents in treatment and control (Equation 1). Hot water extraction of water-soluble nutrients was performed on samples incubated for 30 days. Samples received 20 mL of DI water and were placed in a hot water bath for 1h with occasional shaking. After, samples were shaken for 1h, centrifuged (10,000 g for 10 min) and passed through 0.45-μm syringe filter to remove particulate material. Dilution with 3% nitric acid (HNO_3) followed, after which samples were analyzed by ICP.

$$\text{Equation 1: \% Rednit} = ((NO_3^- \text{-} N_{treatment} - NO_3^- \text{-} N_{control})/ NO_3^- \text{-} N_{control})) \times 100$$

2.3 Statistical analyses

All experiments were evaluated using R statistical software (version 3.6.2). We performed analysis of variance (ANOVA), regression analysis and Partial Least Square Regressions (PLSR) on data from day 30 of the incubations. PLSR combines principal component analysis (PCA) and multiple linear regression to maximize covariance between the predictor and response variables. PLSR was chosen for the following reasons: first, our data presents strong collinearity between predictor variables, and second, we have a relatively low number of

observations which could jeopardize model performance. Thus, we determined that PLSR was suitable for this study.

3 Results

3.1 Flocs-driven changes in soil water-soluble nutrients

The influence of flocs on water-soluble nutrients was assessed in 5 different soil types (Fig. 22). Two-way ANOVA concluded that nutrient concentrations were significantly influenced by soil type, floc type, and the interaction of both (Table 14). The main effect of soil type was the largest, indicating that flocs affected nutrients differently in different soil types. Indeed, the effect of flocs on the concentration of water-soluble elements was more pronounced in mineral than in organic soils. In addition, both floc types significantly increased water-soluble P, Ni, Zn, Mn, Mg, Cu, Co, Ca, Fe, and K concentrations compared to control soils. On the other hand, the concentrations of Mo did not follow this pattern and were significantly decreased in floc-amended soils (Chapter II).

Table 14: ANOVA results. Levels of significance: p < 0.05 (*), p <0.01 (**) and p <0.001 (***). NS: not significant.

	Water soluble Ni	Water soluble Al	Water soluble K	Water soluble Ca	Water soluble Mn	Water soluble Co	Water soluble Zn	Water soluble Mg	Water soluble Cu	Water soluble P	Water soluble Mo	Water soluble Fe
Soil type	F=27.71 p=0.000462***	F=146.6 p<2e-16***	F=67.21 p<2e-16***	F=72.31 p<2e-16***	F=153.48 p<2e-16***	F=70.4 p<2e-16***	F=3.73 p=0.010402*	F=41.95 p=1.23e-14***	F=27.55 p=1.35e-11***	F=139.92 p<2e-16***	F=1260.64 p<2e-16***	F=173.41 p<2e-16***
Floc type	F= 29.46 p=0.000638***	F=282 p<2e-16***	F=3.31 p=0.04561*	F=16.7 p=3.74e-06	F=119.73 p=<2e-16***	F=36.28 p=4.14e-10	F=8.22 p=0.000903**	F=17.50 p=2.37e-06***	F=6.72 p=0.00279**	F=4.10 p=0.023*	F= 102.83 p<2e-16***	F=95.85 p<2e-16***
Interaction effect	F=8.15 p=5.2e-05***	F= 77 p<2e-16***	F= 4.05 p=0.00107**	F=4.03 p=0.00111	F=55.24 p<2e-16***	F=11.69 p=8.26e-09	F=2.60 p=0.019806*	F=6.01 p=3e-05***	F=1.77 p=0.10757	F=11.797 p=26e-09	F=11.03 p=1.87e-08***	F=51.09 p<2e-16***

Figure 22. Concentrations of water-soluble nutrients in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment (Part 1).
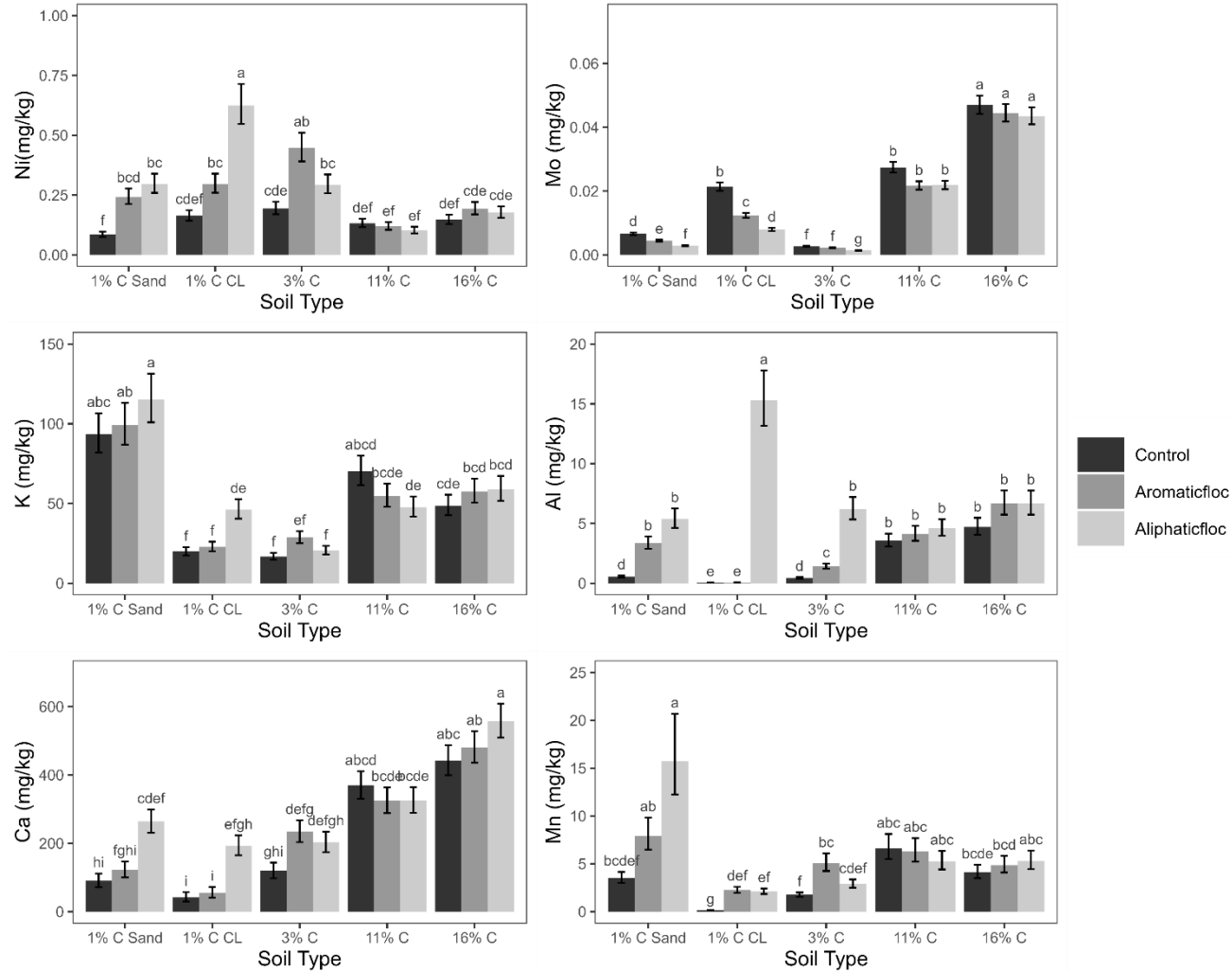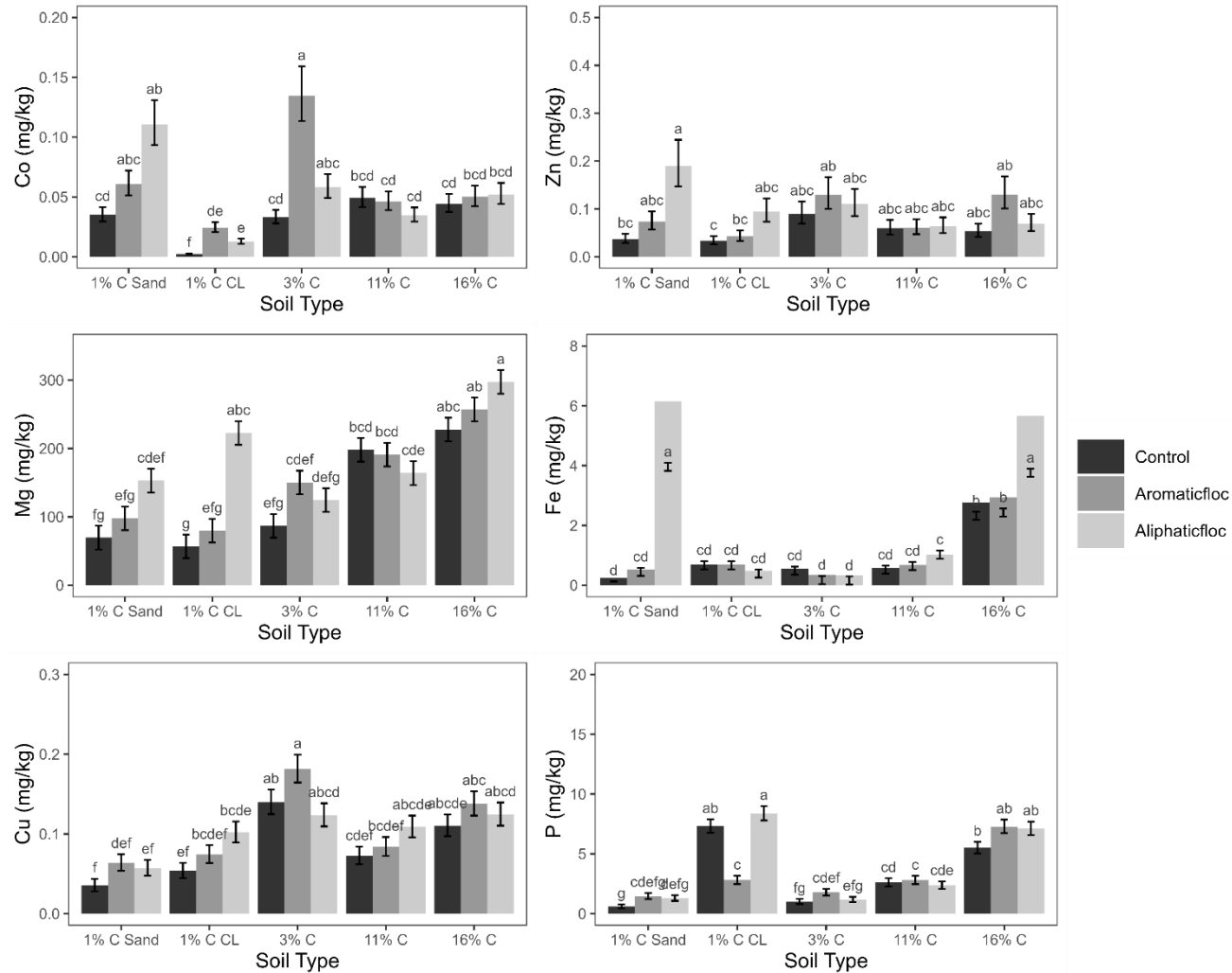
Figure 21. Concentrations of water-soluble nutrients in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment. (Part 2)

3.2 Relationships between Rednit and water-soluble nutrients in soils amended with flocs

We used regression analysis to identify the relationships between Rednit and water-soluble P, Ni, Zn, Mn, Mg, Cu, Co, Ca, Fe, and K concentrations across all soils. Only statistically significant interactions are reported (Figure 23). In this study, the accumulation of Ca, Mg and P positively influence nitrification, whereas Ni, Co, and Mn accumulation increased Rednit across all soils. In contrast, Mo deficiency was negatively related to Rednit across all soils (Chapter II). The remaining elements (Zn, Fe, Al, K, and Cu) did not affect Rednit in this study (Supplemental Fig. S29).

3.3 Relationships between water-soluble nutrients, DOC, and pH in soils amended with flocs

We also used regression analysis to explore the relationships between water-soluble nutrients, DOC, and pH. Of all nutrients, water-soluble Fe exhibited a strong positive correlation with DOC across all soils (Fig. 24). Although weak, relationships between water-soluble Mg, Mn, Al, K, Zn, Ca, and DOC were significant (Fig. 24). No relationships were observed among Co, Zn, Ni, and DOC (Fig. 25). Of all nutrients, only P, Cu and Co had a significant relationship to pH across all soils (Fig. 26, Supplemental Fig. S30).
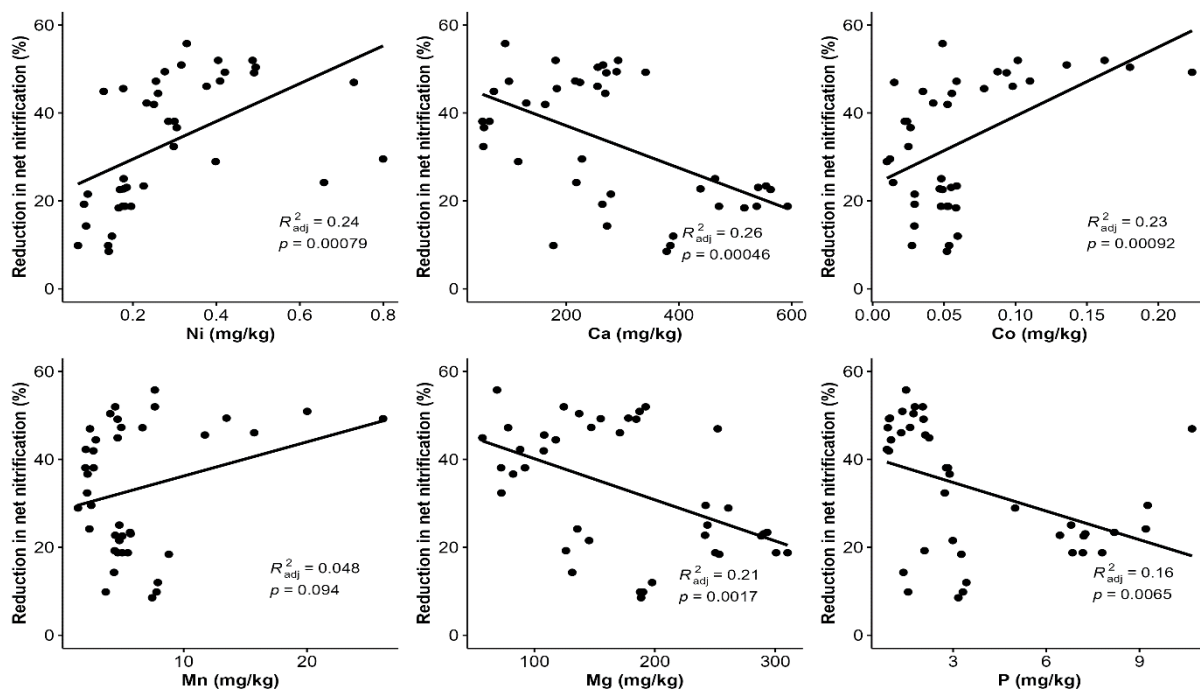
Figure 23. Relationships between Rednit and water-soluble Ca, Mg, P, Ni, Co, Mn, and Mo in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment.
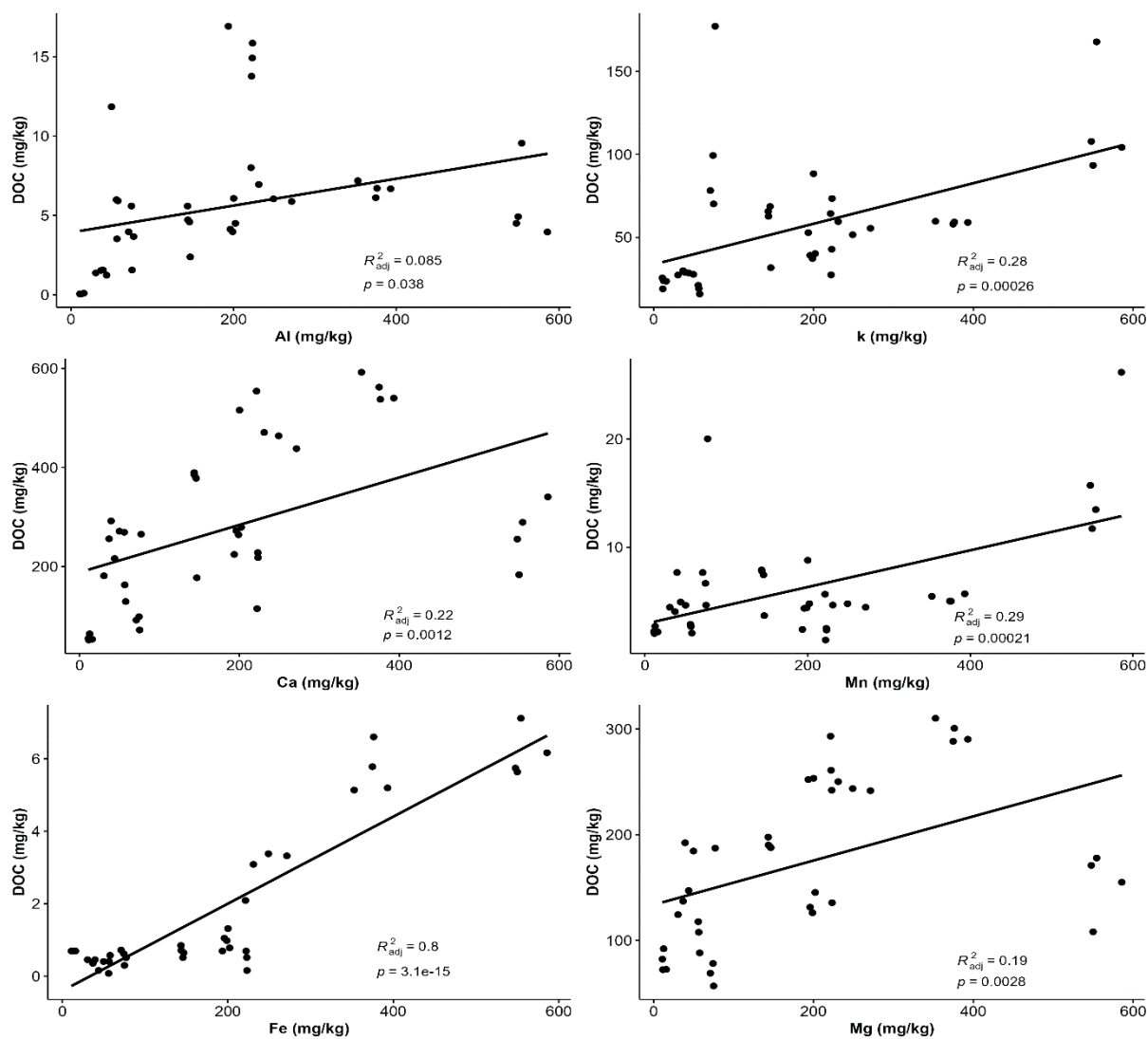
Figure 24. Relationships between DOC and water-soluble Al, K, Ca, Mn, Fe, and Mg in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment.

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Figure 25. Relationships between DOC and water-soluble Co, Zn, and Ni in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment.

Figure 26. Relationships between pH and water-soluble P, Cu and Co in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment.
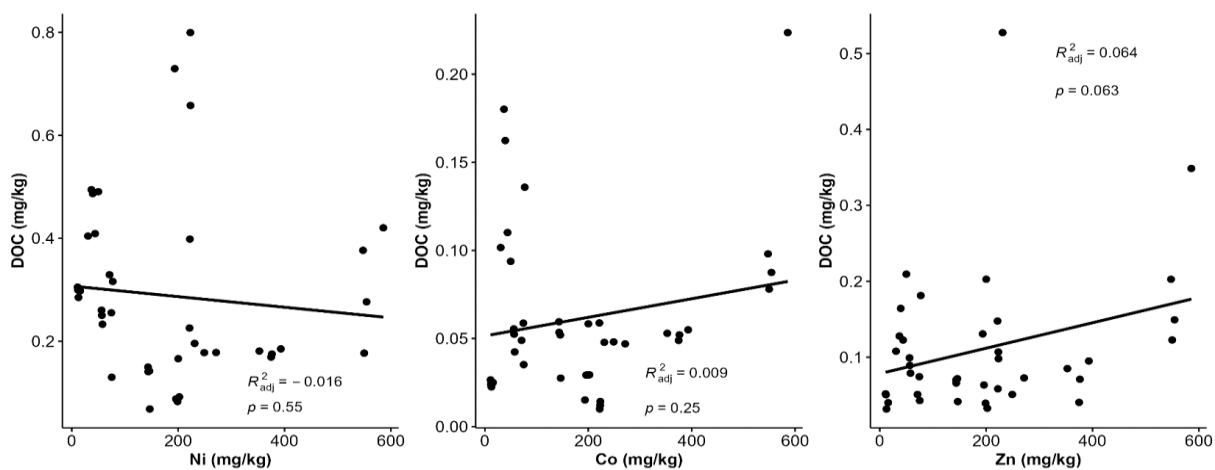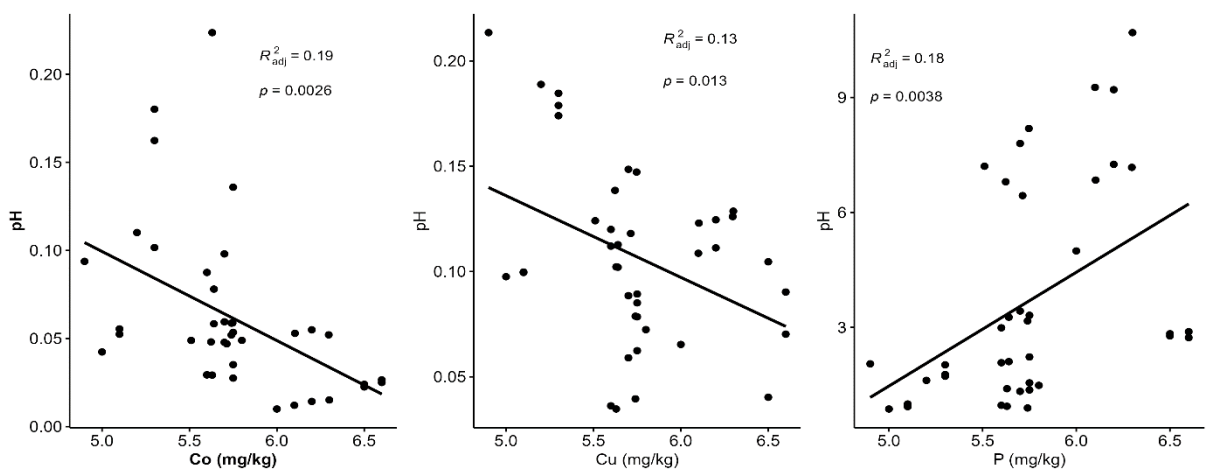
3.4 Rednit in relation to mechanistic drivers in soils amended with flocs

Based on results from PLSR analyses, Rednit in soils amended with flocs can be described as co-varying in its possible mechanistic drivers, such as soil characteristics (e.g., clay and sand content), total DOC and Fe measured by the sum of DOC and Fe originating from both floc and soil, as well as changes in water-soluble nutrients (Figure 27). Our results reveal that including Mo in a predictive model more accurately represented Rednit than the same predictive model with Mo omitted. The percent variability in Rednit explained by the second component of PLSR model dropped by 10% when omitting Mo from the model. The PLSR model with and without Ni increased and decreased, respectively, the percent variability in Rednit explained by 3% on the first and the second components of PLSR). Excluding Co from the PLSR model improved model performance on the second component of PLSR.
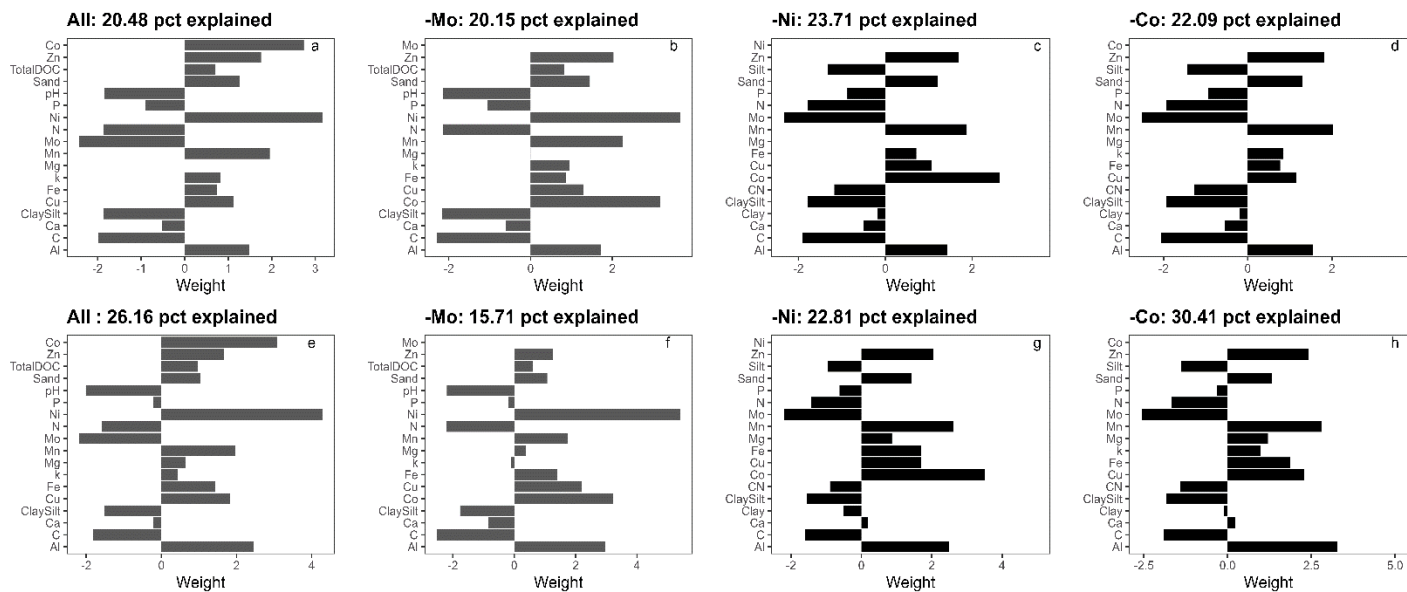
Figure 27. Results of Partial Least Squares Regression Analysis for the mechanistic drivers of Rednit in soils amended with flocs. Component 1 x weights (top row) and component 2 (bottom row) were calculated considering all the mechanistic drivers (a), and omitting Mo (b), Ni (c) and Co (d).

4 Discussion

4.1 Floc-induced change in soil nutrient status: origin, trend, and influence on nitrification

Besides decreasing water-soluble Mo concentrations in both organic and mineral soils (Chapter II), both aromatic and aliphatic flocs increased water-soluble Ca, P, Mg, Ni, Co, Mn, Cu, Zn, Al, and K concentrations in mineral but not in organic soils. This change in soil nutrient status likely influences nitrification, considering the high sensitivity of nitrifying communities to nutrient levels and non-growth substrates (Killham, 1990; Daims et al., 2016; Koch et al., 2019; Fujitani et al., 2020), which has qualified them to be the basis of biological toxicity screening tests of wastewater (Arvin et al., 1994; Dalzell et al., 2002). In soils, research on nitrification has rarely considered the impact of nutrients other than NH_4^+, However, organic amendment-driven changes in cation exchange capacity (CEC) can affect nitrification in soils (Smith, 1964). Therefore, it is important to consider the influence of soil nutrient status on nitrification, especially because when present in excess, most nutrients become toxic to microbial communities.

A previous study (Chapter II) showed that both flocs reduced net nitrification, but the magnitude of their effect was more pronounced in mineral than in organic soils. Regression analysis between water-soluble nutrients and Rednit across all soils showed that the accumulation of Ni, Co, and Mn was positively related to Rednit, whereas opposing relationships between Ca, Mg, P, Mo and Rednit was observed. The first three elements are toxic compounds to nitrifying communities, as shown in both pure culture and soil studies (Loveless and Painter, 1968; Çeçen et al., 2010; Bhunia, 2014). For instance, excess Ni can extend the lag phase of *Nitrosomonas europaea* grown in a medium (Sato et al., 1986) and cause NO_2^- accumulation (Aslan and Sozudogru, 2017). High Ni levels were also shown to inhibit nitrification in a sandy soil (Giashuddin and Cornfield, 1979). Similarly, excess Co restricts both the formation and the oxidation of NO_2^- by nitrifiers grown in a cultivated medium (Loveless and Painter, 1968). Mn toxicity can also retard or fully inhibit nitrification (Xin et al., 2016), especially in soils with

low pH (Hue et al., 2001). Xin et al., (2016) found that the application of Mn oxides to soil decreased AMO *amoA* gene copy (Xin et al., 2016). Besides these direct effects on nitrification, excess Ni, Co, and Mn can reduce N mineralization, which eventually decreases the availability of substrate NH_4^+ to the nitrifying communities. For instance, Liang and Tabatabai, (1977) found that the application of Co, Ni and Mn (applied as salts) reduced soil N mineralization by 2-14 %, 7-17%, and 12-26 % , respectively, in four different clay soils (Liang and Tabatabai, 1977).

In contrast to Ni, Co and Mn, the accumulation of Ca, Mg and P in floc-amended soils positively influenced Rednit. These results were similar to those reported by Raglin et al., (2022) who observed a positive influence of soil Ca and Mg on potential nitrification and nitrifier community beta-diversity in soils under long-term manure application to soil (Raglin et al., 2022). This is because of the essentiality of these nutrients to the growth of the nitrifying communities (Meiklejohn, 1952; Van Droogenbroeck and Laudelout, 1967). In wastewater treatment, nitrifiers can use Ca either as a pH buffer (Green et al., 2002) or as a substrate; for instance, 7.14 mg/l of alkalinity as $CaCO_3$ is needed for the oxidation of one milligram per liter of NH_3 (Bhunia, 2014). Liming was also shown to increase soil NO_3^- (Clough et al., 2004) and provide optimum conditions for N and P mineralization (Curtin et al., 1998; Bolan and Hedley, 2003; Bolan et al., 2003) . Mg promotes the activity of the enzyme succinyl-CoA synthetase responsible for energy (ATP) production in *Nitrosomonas Europeae* (Kondo et al., 1995). A deficiency in P was shown to interfere with ammonia-oxidizing capacity and NO_2^- oxidation (Aleem and Alexander, 1960; Nowak et al., 1996). Flocs also reduced water-soluble Mo across all soils (Chapter II). Mo deficiency is problematic to   nitrifying communities, since besides being an essential micronutrient (Zavarzin, 1957; Finstein and Delwiche, 1965; Tandon et Mishra, 1968), it is used as cofactor in the oxidation of NO_2^- to NO_3^- (Lancaster et al., 2018). Therefore, a deficiency in Mo can depress nitrification in soils.

In contrast to our expectations, we found no relationship between Rednit and Zn, Fe, Al, K, and Cu. However, the toxicity of these elements to nitrification is well documented in the literature. For instance, Cu alters both

nitrification and N mineralization (Liang and Tabatabai, 1977; Sato et al., 1986), especially in low pH soil (Bhunia, 2014). Cela and Sumner, (2002) determined that more than 3.8 mg/kg of water-soluble Cu inhibited soil nitrification, whereas less than 2 mg/kg was tolerable. Similarly, Zn > 0.125 mg/L induced a partial inhibition of nitrification, whereas more than 0.5 mg/L were needed to completely inhibit nitrification (Cela and Sumner, 2002). Both Cu and Zn are more inhibitory to ammonia oxidizers than to nitrite-oxidizing microorganisms. It was also found that supplementing soil with Fe can inhibit nitrification directly (Huang et al., 2016) or by decreasing N mineralization rates (Liang and Tabatabai, 1977). However, Fe can also stimulate nitrification; For instance, Meiklejohn et al., (1952) showed that Fe, at a rate of 6 mg/l, accelerated NH_3 oxidation activity of both *N. europaea* and *Nitrobacter winogradskyi* in pure culture.

Nutrient availability in soils is driven by many factors, including pH, buffering capacity and DOC levels. A previous study (Chapter II) found that both floc types decreased pH in mineral soils. Moreover, the aliphatic floc released Fe and C (as DOC) to soil solution while the aromatic floc did not. While flocs may have contributed to the water-soluble nutrient pool in mineral soils, regression analysis showed that DOC was more important than pH in influencing the concentration of soluble nutrients (Fig. 24 and Supplemental Fig. S28). When pH drops, H^+, as well as the hydrolysed forms of Fe and Al can occupy cation exchange sites and release other cations in soils. However, the fact that increased water-soluble nutrients in mineral soils was accompanied by increased DOC suggests that the solubility of nutrients is promoted by chelation with DOC. Chelating agents can originate from floc decomposition in soils, for instance, the decomposition of the aliphatic floc made carboxymethylcellulose (CMC) can release glucuronic acid, which is an effective chelator of cations in soils. In contrast, anions like Mo can be incorporated into or adsorbed to floc or its decomposition products (Fe and DOC). This adsorption is likely when soil pH decreases (Chapter II).

With some exceptions, water-soluble nutrients were not statistically different in organic soils amended with flocs compared to controls. For Mo, this result can be explained by the fact that OM can enhance the supply of Mo to

soil by releasing Mo tied up in its structure and decrease the adsorption capacity of floc by occupying the adsorption sites and/or causing the microaggregation of Fe oxides. For soil cations, the chelation capacity of flocs may have been counteracted by the high OC content of organic soils. We also posit that nutrients originating from flocs themselves may have adsorbed to negatively charged OM in organic soils.

4.2 Towards a conceptualization of the effects of flocs on nitrification in mineral soils

Summing the effects of soil nutrients on nitrification is complex because multi-element effects on microbes are usually not additive and their interaction may include synergy, additivity, or antagonism (Gikas, 2008). Here, we discuss possible theories on how floc-driven changes in soil nutrient status may affect nitrification.

4.2.1 Theory 1: floc-mediated nitrification in light of Liebig's Law of the Minimum

The effects of flocs-induced changes in soil nutrient status on nitrification in soils can be explained using the concept of Liebig's Law of the Minimum. This law states that the nutrient which is present in its minimum concentration can affect and regulate plant growth. This law applies to microbial processes as well, including nitrification. The growth of nitrifiers, like all microbes, can be described by the following relationship (carbon + nitrogen + phosphorus + other nutrients (e.g., Mo, Fe, Cu, etc.) -> microbial biomass). In a previous study (Chapter II), we found that the bioavailability of Mo was decreased in flocs-amended soils. Considering importance of Mo for both nitrifier growth and activity, as backed up by our PLSR analyses, we posit that Mo was the limiting nutrient for nitrification in mineral soils (Fig. 28).
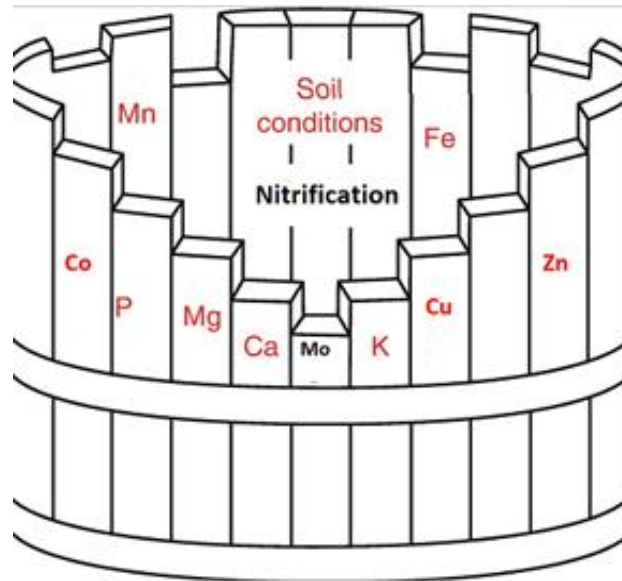
Figure 28. Liebig's Law of the Minimum to illustrate the possible effects of floc-driven changes in soil nutrient status on nitrification.

4.2.2 Theory 2: Nutrient toxicity

Nitrifying communities are very sensitive to changes in soil nutrient status and metal toxicity. Except for Mo, the water-soluble fraction of each element   increased in soils amended with flocs compared to unamended soil. Water-soluble concentration is generally used as indicator of metal toxicity in wastewater. Excess Ni and Co can induce toxicity to nitrifying communities; for instance, Ni toxicity to nitrifiers is possible at the pH of soils amended with flocs (pH= 5.5, Chapter II). However, the lack of established thresholds specific to these microbes in soils makes this statement inconclusive. Besides this, variations in the PLSR model performance suggest that element toxicity is likely mitigated by interactions with other soil elements. For example, Loveless and Painter (1986) found that Co toxicity to nitrifiers in a medium was alleviated by Ca and Mg. Other examples from the literature include the joint positive effects of the application of B and Mo on the size of the nitrifying bacterial population and nitrification rates (Sun et al., 2013), but these benefits can be supressed by the presence of Fe and

Ca, which can complex B and Mo, thereby reducing their bioavailability (Arunkumar et al., 2018; Sun and Selim, 2019). While nutrient toxicity is a possible pathway by which Fe-OC flocs affected Rednit in soils, more studies are needed to decipher these interactions.

4.3 A look forward

This study combined experimental data and a review of the literature to create a conceptual framework of the effects of flocs on nitrification in soils. Flocs appear to affect nitrification in mineral soils by changing soil nutrient status, such as by decreasing the availability of Mo and increasing the availability of Ni, Mg, Ca, P, Mn, Co, and others. This change in soil nutrient status following the input of flocs to soils can be interpreted in terms of both nutrient limitation and toxicity, especially since the addition of Mo to flocs-amended soil did not restore nitrification (Chapter II). We propose that on one hand, nitrification was limited by Mo, as Mo can be adsorbed to flocs or its decomposition productions and/ or incorporated into their structure. On the other hand, nitrification was probably also limited by nutrient toxicity. Given the high sensitivity of nitrifiers to nutrient levels, both nutrient limitation and toxicity are valid reasons for decreased nitrification in soils amended with flocs. Although a clear line on the relative importance of each pathway was not drawn in this study, PLSR analyses showed that both Mo and Ni are important drivers of Rednit. But no clear conclusions can be derived at this time since a threshold concentration of Ni for soil nitrifiers has not been established in soils.

To date, research has identified NH_4^+ and pH as the most important factors regulating soil nitrification. However, this study sheds light on the importance of soil micronutrients in this process. Indeed, Raglin et al. (2022) showed that the long-term accumulation of specific soil cations changes the nitrifier community composition which eventually influences nitrification. These authors also mentioned that soil nutrients may seem irrelevant in the short term but can emerge as important drivers of nitrifier distribution and function in the long term.

Because floc model compounds can limit nitrification and extend the duration of NH_4^+ in agricultural soils (Chapter II), data on floc-driven changes in soil nutrient status is also useful to understand their possible effects on plant growth and development. In general, a deficiency in one or more soil nutrients can decrease plant growth and development (Merchant, 2010), or can cause toxicity when present in excess (Juliastuti et al., 2003; Jacob and Otte, 2004; Eid et al., 2012). However, a study by Liang et al. (2019) found that coagulation treatment with aluminum (Al)- and Fe-based coagulants did not disrupt the growth of Typha plant grown in a constructed wetland, despite a slight change in soil nutrient status within a two-year period. More studies are needed to explore the usefulness of using flocs in agriculture or as part of a wetland restoration strategy, especially in terms of their effects on both plant growth and other N cycling transformations, such as denitrification.
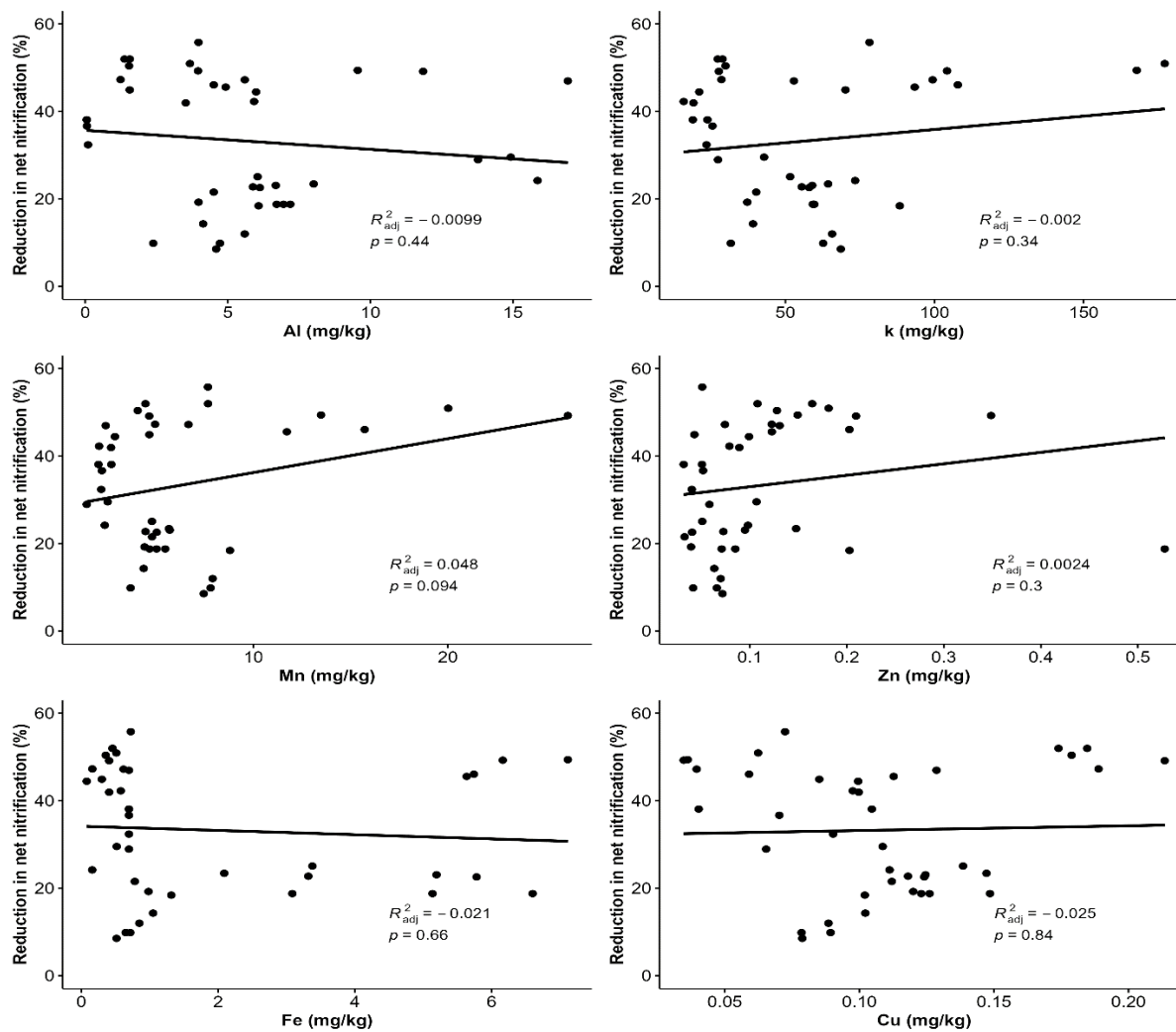
Supplemental Figure S29. Relationships between Rednit and water-soluble Zn, Fe, Al, K, and Cu in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment.
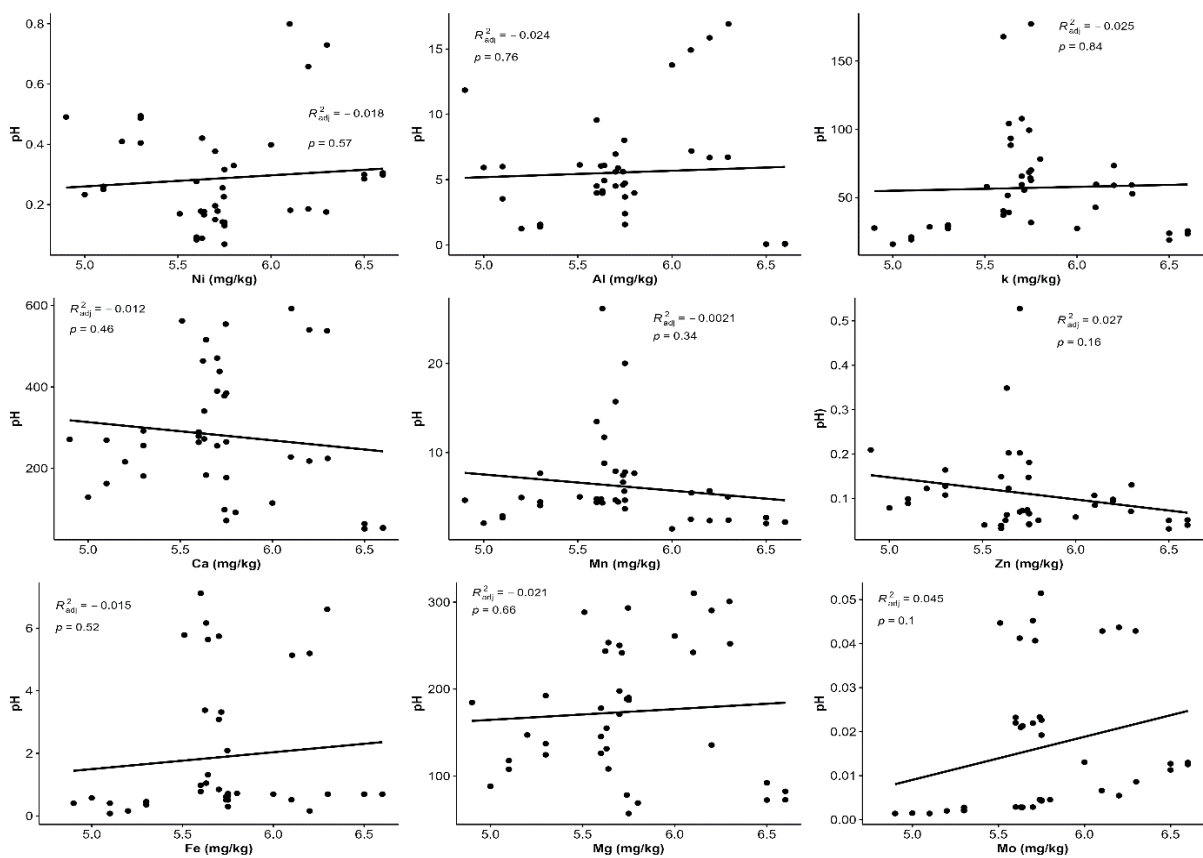
Supplemental Figure S30. Relationships between pH and water-soluble nutrients in soils amended with aliphatic and aromatic flocs, calculated after 30 days of incubation. Values are averages of 4 repetitions per treatment.

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