

REVIEW

Nitrogen loading enhances phosphorus limitation in terrestrial ecosystems with implications for soil carbon cycling

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Abstract

- Increased human-derived nitrogen (N) loading in terrestrial ecosystems has caused widespread ecosystem-level phosphorus (P) limitation. In response, plants and soil micro-organisms adopt a series of P-acquisition strategies to offset N loading-induced P limitation. Many of these strategies impose costs on carbon (C) allocation by plants and soil micro-organisms; however, it remains unclear how P-acquisition strategies affect soil C cycling. Herein, we review the literature on the effects of N loading on P limitation and outline a conceptual overview of how plant and microbial P-acquisition strategies may affect soil organic carbon (SOC) stabilization and decomposition in terrestrial ecosystems.
- Excessive input of N significantly enhances plant biomass production, soil acidification, and produces plant litterfall with high N/P ratios, which can aggravate ecosystem-level P limitation.
- Long-term N loading can cause plants and soil micro-organisms to alter their functional traits to increase P acquisition. Plants can release carboxylate exudates and phosphatases, modify root morphological traits, facilitate the formation of symbiotic associations with mycorrhizal fungi and stimulate the abundance of P-mineralizing and P-solubilizing micro-organisms. Releasing carboxylate exudates and phosphatases could accelerate SOC decomposition, whereas changing symbiotic associations and root morphological traits (e.g. an increase in fine root length) may contribute to higher SOC stabilization. Increased relative abundances of P-mineralizing and P-solubilizing bacteria can accelerate P mining and SOC decay, which may decrease microbial C use efficiency and subsequently lower SOC sequestration.

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4. The trade-offs between different plant P-acquisition strategies under N loading should be among future research priorities due to their cascading impacts on soil C storage. Quantifying ecosystem thresholds for P adaption to increased N loading is important because P-acquisition strategies are effective when N loading is below the N threshold. Moreover, understanding the response of P-acquisition strategies at different levels of native soil N availability could provide insight to divergent P-acquisition strategies across sites and ecosystems. Altogether, P-acquisition strategies should be explicitly considered in Earth System Models to generate more realistic predictions of the effects of N loading on soil C cycling.

KEY WORDS

carboxylate exudation, extracellular enzyme activity, nitrogen loading, phosphorus limitation, phosphorus-acquisition strategies, symbiotic association

1 | INTRODUCTION

Nitrogen (N) and phosphorus (P) are two of the most important limiting factors of plant primary production and microbial functions in the majority of terrestrial ecosystems (Du et al., 2020; Elser et al., 2007; Hou et al., 2020). Human activities have substantially altered ecosystem N and P dynamics, which have changed the pattern and extent of global nutrient limitations (Vitousek et al., 2010). Of particular importance is the increase in anthropogenic N loading since the Industrial and Green Revolutions (Peñuelas et al., 2013; Yu et al., 2019). Fossil fuel combustion and agricultural fertilization have more than doubled natural levels of reactive N inputs to terrestrial ecosystems, in the forms of reduced (e.g. ammonium, ammonia), oxidized (e.g. nitrate, nitric oxide, nitrous oxide), or, to a lesser extent, organic N (Galloway et al., 2008; Peñuelas et al., 2020). However, sources of P in terrestrial ecosystems are mainly from rock and mineral weathering, dust transport and atmospheric P deposition (Walker & Syers, 1976; Zhu et al., 2016). In contrast to the large amounts of anthropogenic N loading, the input of P is relatively small and much less readily available to plants and soil micro-organisms (Hou et al., 2021; Peñuelas et al., 2013; Vitousek et al., 2010; Wang et al., 2022). These unbalanced N and P inputs have resulted in widespread ecosystem-level P limitation (Ding et al., 2021; Hou et al., 2020; Li et al., 2016; Vitousek et al., 2010). Indeed, evidence of P limitation caused by N loading has been widely reported in a variety of terrestrial ecosystems, including tropical forests, grasslands, wetlands and tundra (Deng et al., 2017; Du et al., 2020; Hou et al., 2021; Li et al., 2016).

Soil is one of the largest carbon (C) reservoirs in terrestrial ecosystems, and soil C storage is commonly influenced by soil nutrient status (Fleischer et al., 2019; Wieder et al., 2015). Increasing soil N availability may impact soil C storage by altering C uptake (e.g. photosynthesis), release (e.g. respiration) and sequestration in both plants and soils (Vitousek et al., 2010). Furthermore, N loading-induced P limitation may affect soil C cycling (Ding et al., 2021). Given that all living organisms seek to maintain a stoichiometric

balance, plants and soil micro-organisms may adopt a series of P-acquisition strategies to offset the effects of N loading-induced P limitation (Chen & Moorhead, 2022; Lambers et al., 2013; Marklein & Houlton, 2012; Tian et al., 2020; Wen et al., 2021). For instance, vascular plants can increase P acquisition by releasing root exudates, forming symbiotic associations with mycorrhizal fungi or modifying root morphological traits (Jian et al., 2016; Vitousek et al., 2010). Cryptogams, such as mosses and lichens, are also well known for their ability to regulate the release of extracellular phosphatases in response to increasing N loading-induced P limitation in ecosystems such as peatlands, bogs and the understorey of boreal forests (Arróniz-Crespo et al., 2008; Ochoa-Hueso et al., 2011). Plants can also increase the relative abundance of P-mineralizing and P-solubilizing free-living micro-organisms (mostly bacteria), which can contribute to mobilization of P through the release of phosphatases and low-molecular-weight organic acid (Chen, Li, et al., 2018; Chen, Luo, et al., 2018; Marklein & Houlton, 2012). These P-acquisition strategies may directly or indirectly affect C allocation by plants or soil micro-organisms because these strategies have C and energy costs (Lugli et al., 2020; Ryan et al., 2012). However, it is unclear how these P-acquisition strategies may affect soil organic carbon (SOC) stabilization and decomposition (Ding et al., 2021). Uncertain impacts of P-acquisition strategies (i.e. P limitation adaptation) on soil C cycling limit our ability to fully understand ecosystem feedback to N loading in the context of coupled C–N–P interactions (Fleischer et al., 2019; Goll et al., 2012; Peñuelas et al., 2013).

In this review, we first summarize how increased N loading may induce ecosystem-level P limitation and potential consequences on ecosystem-level C cycling. We then discuss how plant and microbial adaptation to N loading-induced P limitation may affect soil C cycling. Finally, we identify key research priorities for future study. This review will advance our understanding of how N loading-induced P limitation is linked to soil C cycling in terrestrial ecosystems, and thus will increase our capability to better model coupled C, N and P biogeochemical cycles under global climate change.

2 | EFFECTS OF NITROGEN LOADING ON ECOSYSTEM-LEVEL PHOSPHORUS LIMITATION

2.1 | Increased plant biomass production under nitrogen loading enhances ecosystem-level phosphorus limitation

Increases in plant biomass production with N loading can increase plant and microbial demands on available P as P is needed for stoichiometric balance in both plants and microbes, leading to ecosystem-level P requirements (Figure 1). Previous studies demonstrated that N loading greatly increased plant biomass (Li et al., 2016). For instance, a meta-analysis of 288 sites across the globe showed that N loading increased above-ground and below-ground biomass by 40% and 11%, respectively (Deng et al., 2017). Similarly, another meta-analysis found that P-induced increases in plant above-ground biomass increased with elevated N (Li et al., 2016). Nevertheless, N loading induced greater plant biomass, which leads to greater P demand as P is a non-substitutable structural component of deoxyribonucleic acid, ribonucleic acid, adenosine triphosphate, phospholipids and other components in plant cells (Jiang et al., 2019). In addition, N loading-induced P limitation is indirectly supported by a positive relationship between plant biomass and soil phosphatase activities under N addition across various terrestrial ecosystems (Chen, Van Groenigen, et al., 2020), because phosphatase activity is widely considered as a proxy for ecosystem-level P limitation (Marklein & Houlton, 2012). Overall, increased plant biomass production could be an important driver of ecosystem-level P limitation in terrestrial ecosystems under N loading.

2.2 | Soil acidification under nitrogen loading can increase or decrease ecosystem-level phosphorus limitation

Nitrogen loading can increase soil acidification by increasing proton release from nitrification, nitrate uptake and nitrate leaching (Lu et al., 2014). A meta-analysis suggested that N loading significantly reduced soil pH by an average unit of 0.26 in global terrestrial ecosystems (Tian & Niu, 2015). Lower soil pH in neutral and acidic soils could promote the dissolution of aluminium (Al) hydroxides and/or iron (Fe) (oxyhydr)oxides or calcium (Ca) carbonate, increasing exchangeable Al^{3+} , Fe^{3+} and Ca^{2+} in the soils (Lu et al., 2014). These cations can accelerate the formation of iron, aluminium and calcium bound phosphates, reducing soil phosphate availability for plant and microbial uptake (Deforest & Scott, 2010). According to the 'adsorption-penetration theory' (Barrow, 2021), there would likely be an increased in the adsorption reaction between phosphate ions and the surface of iron or aluminium hydroxides or (oxyhydr)oxides and clay minerals, reducing phosphate availability. Both mechanisms of N loading-induced soil acidification and associated soil P precipitation and adsorption will aggravate ecosystem P limitation (Zhou, Lu, et al., 2018).

In contrast to neutral and acidic soils, N loading on alkaline soils, including arid or semi-arid ecosystems (drylands) may not induce serious soil acidification, because the buffering capacity of alkaline soil is high (Zhong & Zhou, 2022). Furthermore, studies found that the effects of N loading on ecosystem P limitation are variable in alkaline dryland soils (Cook et al., 2018; Kooijman et al., 2020). For instance, Wang et al. (2020) found that N addition increased ecosystem P limitations in grasslands of China, because increased N can enhance the mobilization of Al to form Al mineral bound phosphates. However,

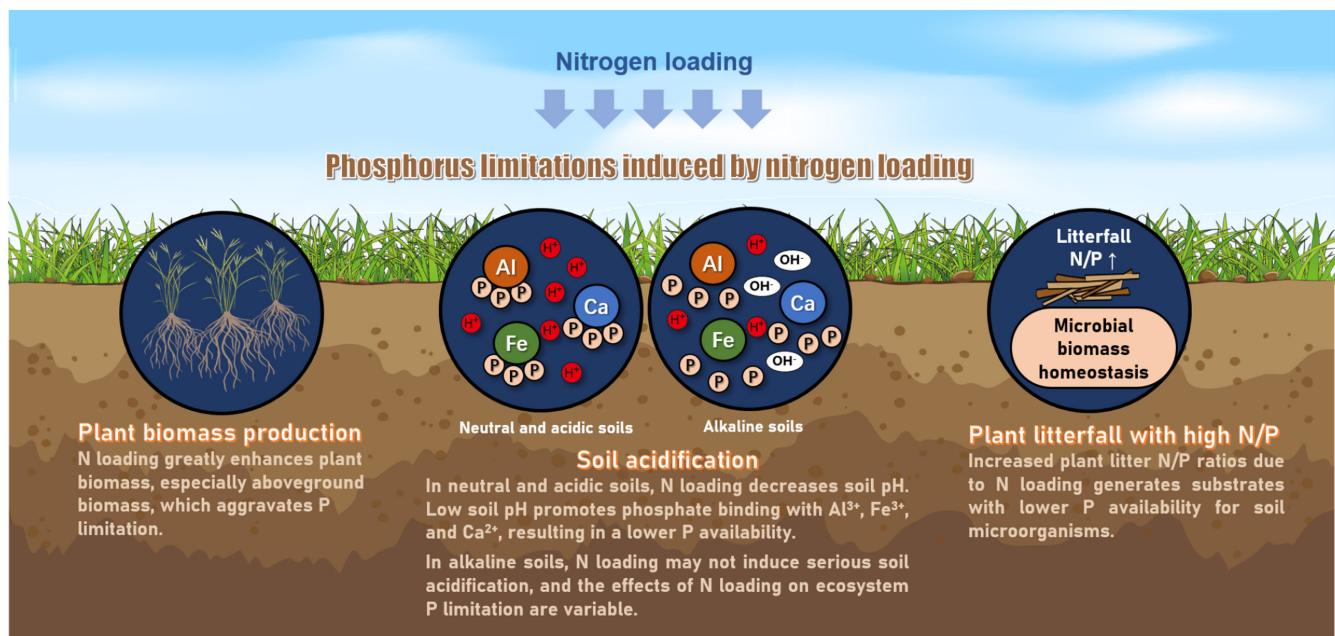


FIGURE 1 Conceptual diagram depicting the effects of nitrogen loading on plant biomass production, soil acidification and plant litters with high nitrogen/phosphorus ratios, with cascading impacts on enhancing ecosystem-level phosphorus limitation.

Zuccarini et al. (2021) found that short-term N deposition in a Mediterranean shrubland (Spain) alleviated P limitation by increasing both extractable P and the activities of P-acquiring enzymes.

2.3 | Plant litterfall with high nitrogen/phosphorus ratios elevate ecosystem-level phosphorus limitations under nitrogen loading

Nitrogen loading could accelerate ecosystem P limitation by generating large amounts of plant litterfall with high N/P ratios. Nitrogen loading increases plant biomass and thus elevates the quantities of plant litterfall (Hou et al., 2021). In addition, N loading increases N concentrations in plants, consequently changing the litterfall quality (i.e. increasing litterfall N/P ratios, Fan et al., 2018; Heuck et al., 2018). Overall, N loading produces substrates with high N/P ratios for soil micro-organisms (Figure 1). However, most soil micro-organisms tend to maintain biomass element homeostasis (i.e. microbial biomass N/P remains relatively unchanged) (Zhang et al., 2019). This is because soil micro-organism element homeostasis is relatively independent of external conditions and has a relatively large tolerance to variations in ecological factors (Sterner & Elser, 2002). As a result, litterfall of high N/P ratios due to N loading likely reduces P availability relative to N availability, which may reduce net P mineralization rates from decomposition that increases ecosystem-level P limitations (Fleischer et al., 2019; Li et al., 2016).

3 | PLANT PHOSPHORUS-ACQUISITION STRATEGIES AND THEIR CASCADING IMPACTS ON SOIL CARBON CYCLING

To adapt to enhanced N loading and associated P limitation, plants can shift their physiological, morphological and symbiotic mycorrhizal traits to increase P acquisition (Ding et al., 2021; Pang et al., 2018; Wen et al., 2021). Considering the central role of plants in soil C cycling, we discuss how plant P-acquisition strategies potentially alter SOC stabilization and decomposition.

3.1 | Roles of carboxylate exudation in soil carbon cycling

A highly efficient P-acquisition strategy for plants is to release carboxylates from roots (Lambers et al., 2013, 2015; Wen et al., 2021) because P limitation induced by N loading favours the allocation of plant resources towards root exudation (Li et al., 2018; Zhu et al., 2016) and carboxylates (e.g. citrate, malate and malonate) are the major components of root exudates, especially in P-deficient soils (Delgado et al., 2014; Ding et al., 2021).

Such an N loading-induced carboxylate exudation may have a significant influence on soil C cycling (Figure 2a). However, the

underlying mechanisms differ between acidic and alkaline soils. In acidic soils, carboxylates can liberate C from mineral-organic complexes via destabilization and dissolution under N loading (Figure 2a; Pang et al., 2018). Moreover, releasing carboxylates and other low-molecular-weight C compounds into the soil can stimulate the priming effect under higher N loading (Keiluweit et al., 2015; Shahzad et al., 2015) because carboxylates are easily assimilable organic matter that can serve as a co-metabolite (Kuzyakov & Xu, 2013). Therefore, carboxylate exudation enhanced by N loading has the potential to increase SOC decomposition and thus foster the release of more CO₂ in acidic soils (Figure 2a). In alkaline dryland soils, inorganic C dominates the C pool (Lal, 2019) so that enhanced carboxylate exudation causes dissolution of calcium phosphates (Ding et al., 2022). The mobilized Ca²⁺, in turn, could precipitate with CO₂ and H₂O derived from SOC decomposition or root and microbial respiration to form secondary pedogenic carbonates (Zhong & Zhou, 2022). Although drylands typically have low soil organic matter, they comprise the world's largest biome (Cook et al., 2018). Thus, N loading-induced carboxylate exudation in alkaline dryland soils may promote soil inorganic C sequestration, which could play an important role in global C biogeochemical cycles (Figure 2b).

In the future, more laboratory and field data are required for validating the potential role of carboxylate exudation in soil C dynamics. Both ¹³C and ¹⁴C isotope-labelled carboxylates could be used to explore the role of carboxylates on soil C storage in laboratory studies (Ding et al., 2022). In field studies, it is difficult to accurately determine concentrations of carboxylates in the rhizosphere (Zhou et al., 2021). However, the manganese (Mn) content in leaves could be used as an index of rhizosphere carboxylate concentrations as high leaf Mn contents reflect high concentrations of carboxylates in the soil (Lambers et al., 2015).

3.2 | Potential impacts of changing root morphological traits on soil carbon cycling

Nitrogen additions have been reported to decrease root biomass and specific root length and root surface area (Chen, Tu, et al., 2017; Wang et al., 2013). One explanation might be that increased soil N availability will induce plants to allocate a lower proportion of biomass into below-ground organs based on costs versus benefits, in agreement with the optimal allocation hypothesis (Chen, Tu, et al., 2017; Mei et al., 2010). However, N loading-induced P limitation will cause plants to alter root morphological traits in response to low P supply (Jiang et al., 2019; Lynch & Brown, 2008), such as elevating lateral root branching, increasing the length of fine roots, increasing root hairs and enlarging aerenchyma (Figure 3). For example, a study indicated that N loading increased the fine root biomass, which is a common root morphological change in response to low P concentrations in forest ecosystems (Zhu et al., 2021). Another study suggested that high N application caused similar changes to root morphology as low P availability did in a subtropical *Pinus massoniana* forest (Song et al., 2016). These studies validate the

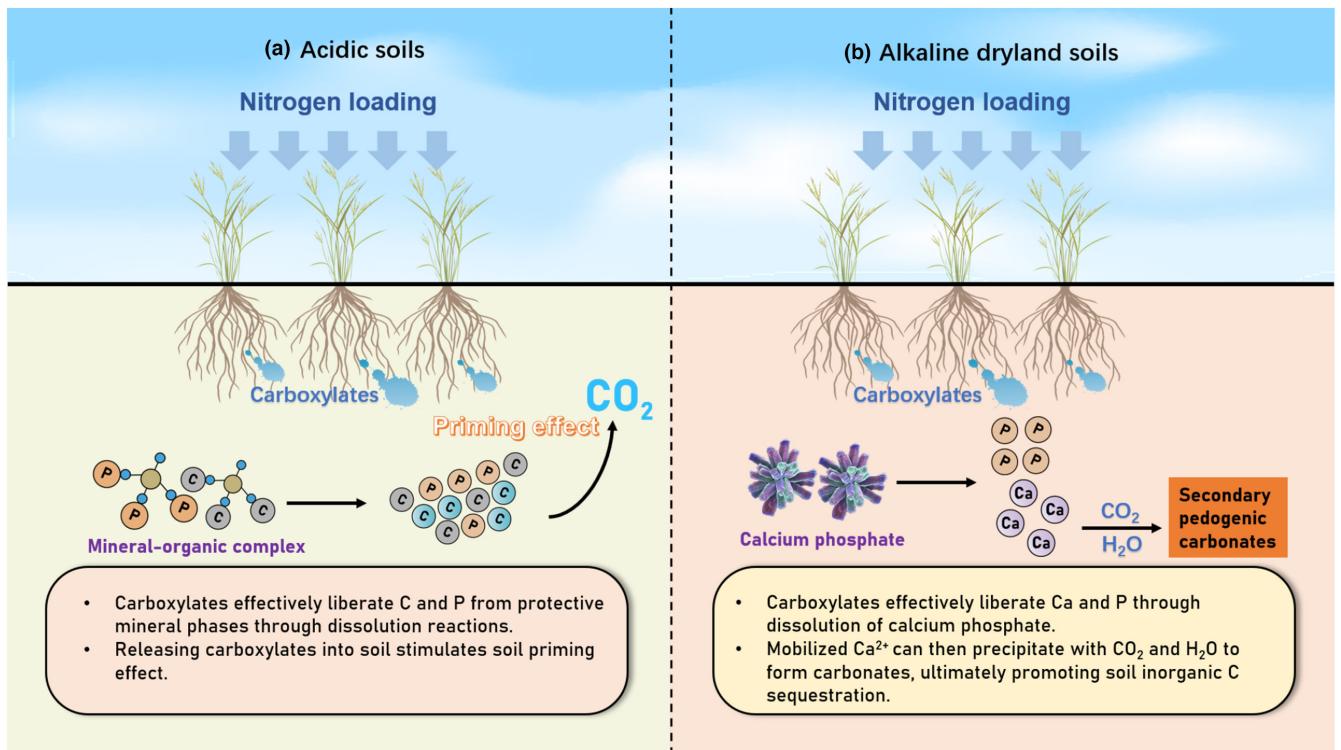


FIGURE 2 Conceptual diagram illustrating the mechanisms underlying effects of nitrogen loading-induced carboxylate release on soil carbon cycling in (a) acidic soils and (b) alkaline dryland soils. In both soils, nitrogen loading will stimulate plants to release carboxylates to acquire more phosphorus from soils. In acidic soils, carboxylate exudation will effectively liberate carbon through dissolution of mineral complexes. Furthermore, carboxylate exudation will accelerate soil priming effect because carboxylates contain easily assimilable carbon that can serve as a co-metabolite. Thus, carboxylate exudation has the potential to increase soil organic carbon decomposition and release carbon dioxide. In contrast, carboxylate exudation in alkaline dryland soils results in dissolution of calcium phosphates. The mobilized calcium ions (Ca²⁺) may then precipitate with CO₂ and H₂O to form secondary pedogenic carbonates, ultimately promoting soil inorganic carbon sequestration.

hypothesis that P limitation due to N loading could alter root morphological traits.

Changes in root morphological traits associated with N loading-induced P limitation can affect SOC stabilization (Poirier et al., 2018). A high fine root density, extensive fibrous roots and a high root branching index under P limitation induced by N loading can increase soil microaggregates by entangling and enmeshing soil particles (Gould et al., 2016; Poirier et al., 2018), with the occlusion of soil organic matter within soil aggregates recognized as a major mechanism stabilizing SOC (Lützow et al., 2006). In contrast to SOC stabilization, the potential role of changes in root morphological traits associated with N loading-induced P limitation in SOC decomposition has not been sufficiently studied (Poirier et al., 2018). For instance, experiments have shown that increases in fine root length induced by P limitation accelerated SOC decomposition for graminoids (Roumet et al., 2016). However, Poirier et al. (2018) suggested that changes in root morphological traits, such as increases in root length and diameter, were poorly related to rhizosphere SOC decomposition. These inconsistencies may result from not separating morphological traits from root exudates (Ding et al., 2021), which future studies may have to elucidate the effects of changes in root morphology on SOC decomposition.

3.3 | Nitrogen loading-induced symbiotic fungal associations affect soil carbon cycling

Under N loading, plants can enhance P uptake and growth through enhanced associations with mycorrhizal fungi (including ectomycorrhizal and arbuscular mycorrhizal fungi), particularly under low P availability (Lambers et al., 2006; Pang et al., 2018). For instance, it was suggested that ectomycorrhizal fungal activity increased under N application as the soil organic P fraction decreased to meet increased P demand in a subtropical forest ecosystem (Fan et al., 2018). Furthermore, arbuscular mycorrhizal fungi alleviated P limitation by reducing plant N/P ratios under N addition in a temperate meadow ecosystem (Mei et al., 2019). Mycorrhizal associations can stimulate plant P absorption and enhance soil organic P mineralization by releasing organic acids and phosphatase (Zhang et al., 2018). Additionally, mycorrhizal associations can contribute to enhanced P release and uptake by promoting the accumulation of P-solubilizing bacteria in the hyphosphere (i.e. the soil volume affected by fungal hyphae) (Lilleskov et al., 2019; Figure 4).

Mycorrhizal fungi could increase soil aggregation (Leifheit et al., 2015), as fungal hyphae entangle soil particles and stabilize

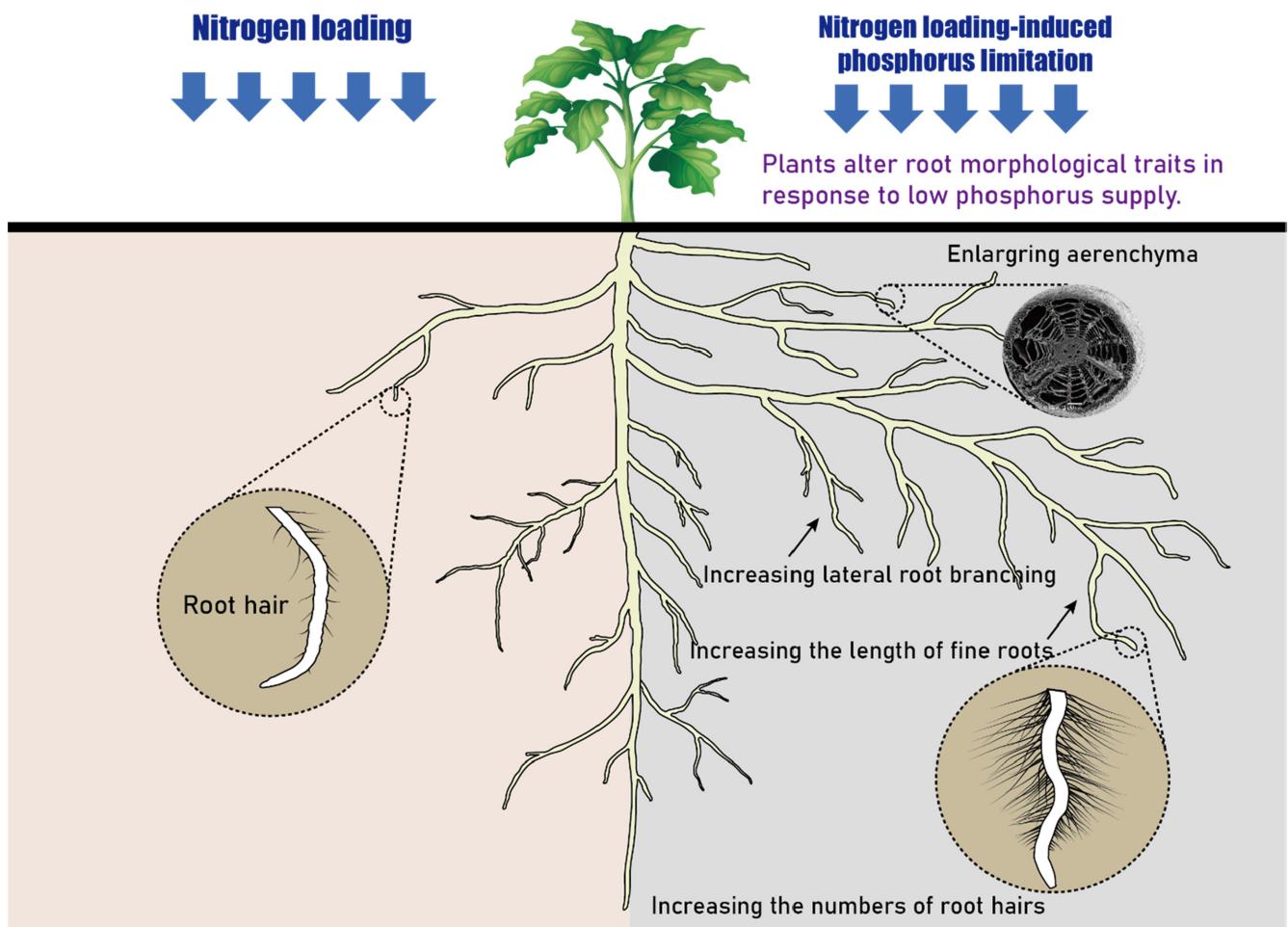


FIGURE 3 Conceptual diagram depicting how plants alter root morphological traits in response to nitrogen loading-induced phosphorus limitation versus the direct effects of nitrogen loading, such as increasing lateral root branching, increasing the length of fine roots, increasing the number of root hairs and enlarging aerenchyma. Increases in lateral root branching and fine root length can increase soil organic carbon stabilization.

macroaggregates, especially in the rhizosphere (Baumert et al., 2021; Witzgall et al., 2021). At the same time, mycorrhizal fungi can also stimulate SOC decomposition by releasing extracellular enzymes and hydroxyl radicals to soil (Cheng et al., 2012). Moreover, mycorrhizal fungi could accelerate the mineralization of initial SOC through releasing carbon subsidies (mycorrhizal exudates and necromass) to the rhizosphere (i.e. priming; Liu et al., 2021). In contrast, mycorrhizal associations can also suppress SOC decomposition by reducing the saprotrophic activity of decomposers under N loading (Zhou et al., 2020), which is referred to as the Gadgil effect (Gadgil & Gadgil, 1971).

Inconsistent effects of mycorrhizal fungi on soil C cycling under N loading-induced P limitation may be due to the diversity of mycorrhizal fungi in the soil and associations with plants (Ding et al., 2021; Qiu et al., 2021). For instance, within arbuscular mycorrhizal fungi, species in the *Gigasporaceae* family were observed to contribute less to SOC stabilization than species in the *Glomeraceae* (Treseder et al., 2018). Future research is needed to investigate effects of different species, community richness and abundance of mycorrhizal fungi on soil C cycling under low-P conditions.

4 | IMPACTS OF MICROBIAL PHOSPHORUS-ACQUISITION STRATEGIES ON SOIL CARBON CYCLING

4.1 | Nitrogen loading-induced enzyme production affects SOC decomposition

Increasing extracellular enzyme activities is a key strategy for plants, plant symbionts and soil micro-organisms to overcome nutrient limitations (Sinsabaugh et al., 2010). To increase P acquisition under N loading, an increase in the production of acid and alkaline phosphatases by P-mineralizing bacteria has been reported in several meta-analyses across a range of terrestrial ecosystems (Chen, Li, et al., 2018; Chen, Van Groenigen, et al., 2020; Deng et al., 2017; Jian et al., 2016; Marklein & Houlton, 2012). One explanation is that under N loading soil micro-organisms can allocate excess N to produce phosphatases, thus reducing P limitation (Allison & Vitousek, 2005). Alternatively, an increased availability of readily accessible N may shift microbial priorities to increase acquisition of P, as predicted by stoichiometric theory (Sinsabaugh & Follstad

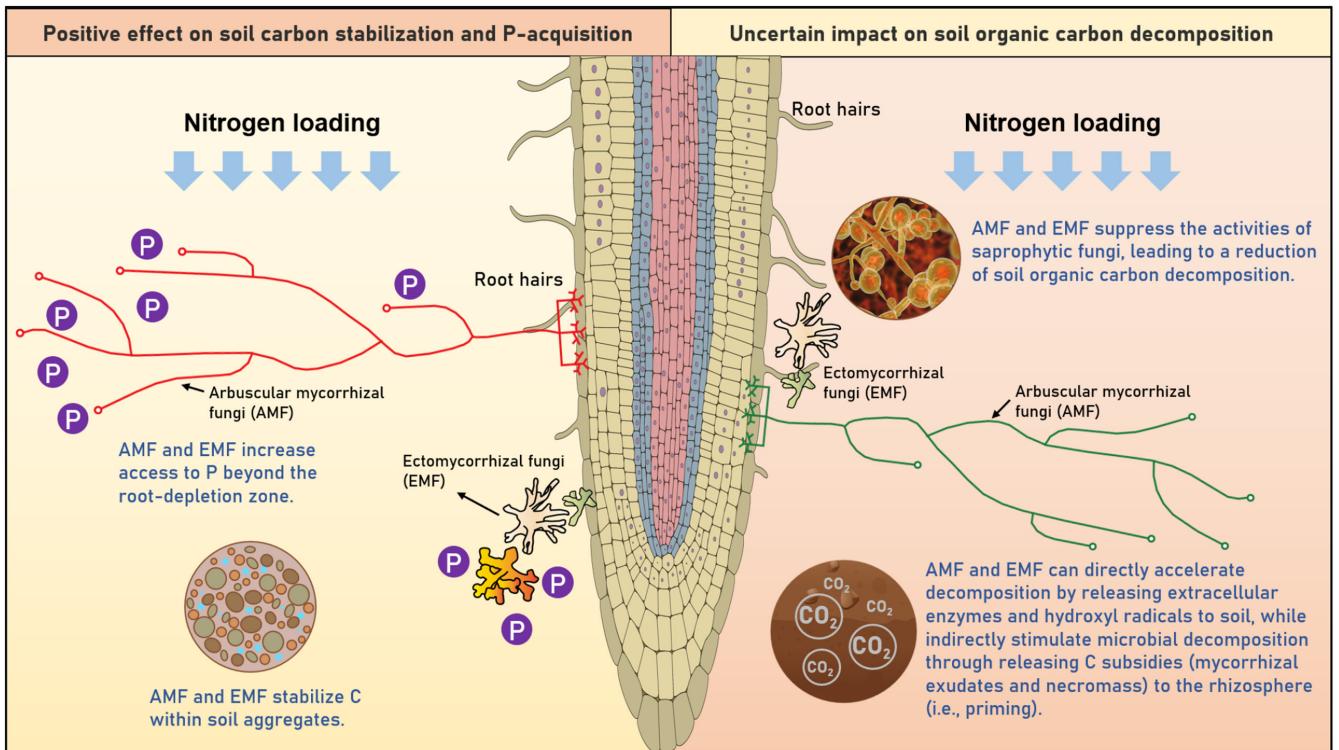


FIGURE 4 Conceptual diagram illustrating effects of ectomycorrhizal (EMF) and arbuscular (AMF) mycorrhizal associations on phosphorus-acquisition and soil organic carbon cycling. Both AMF and EMF could stabilize carbon within soil aggregates, directly accelerate decomposition by releasing extracellular enzymes and hydroxyl radicals to soil, and indirectly stimulate microbial decomposition by releasing carbon subsidies (mycorrhizal exudates and necromass) to the rhizosphere (i.e. priming). Moreover, AMF and EMF can suppress soil organic carbon decomposition by inhibiting saprotrophic activity.

Shah, 2012). In addition to soil micro-organisms, some higher vascular plants and cryptogams (e.g. mosses and lichens) can release phosphatase in response to N loading (Arróniz-Crespo et al., 2008). For example, long-term (7 years) N deposition in a calcareous grassland in the UK increased root surface phosphatase activities for a sedge *Carex flaccca*, a grass *Koeleria macrantha* and a forb *Leontodon hispidus* (Phoenix et al., 2004). In the case of higher vascular plants and mosses, increasing the production of phosphatase may also be considered as a strategy to avoid the highly negative effects of increased N loading on plant physiology (Ochoa-Hueso et al., 2014).

Regardless of the cause (or source), increased soil phosphatase activity has important effects on soil C cycling. Although most soil P is stored in inorganic forms, a portion is stored as organic P consisting mainly of monoesters and diesters (Dijkstra et al., 2013; Marklein & Houlton, 2012). Therefore, soil micro-organisms also need to allocate energy to produce C-acquiring enzymes for the hydrolysis of organic P (Chen, Li, et al., 2018; Chen, Luo, et al., 2018). Indeed, studies have reported that hydrolytic C-acquiring enzyme activity (i.e. glucosidase, cellobiosidase and xylosidase) increased after N loading (Chen, Luo, et al., 2017; Chen, Luo, et al., 2018; Jian et al., 2016). This mainly occurs because N loading enhances relative microbial C limitations (Chen, Li, et al., 2018). However, N loading-induced P limitation can also indirectly stimulate C-acquiring enzyme activity and accelerate SOC decomposition (Figure 5). Nitrogen loading-induced increases in soil phosphatase activity have been reported

in both croplands and natural ecosystems (Chen, Van Groenigen, et al., 2020). However, the proportion of organic P in the total soil P pool is quite different between croplands and natural ecosystems (Hou et al., 2021), which may differently affect the responses of soil phosphatase activity to N loading (Marklein & Houlton, 2012). In the future, more attention should be paid to differences in the phosphatase production mechanisms between croplands and natural ecosystems.

4.2 | Phosphorus-solubilizing bacteria and their roles in SOC decomposition

Another microbial strategy to acquire P under N loading is to increase the abundance of P-solubilizing bacteria (Carrara et al., 2018). Phosphorus-solubilizing bacteria contain abundant P-cycling-related genes (e.g. *phoD*, *phoB* and *phoR*), which can be particularly abundant in P-deficient soils (Keiluweit et al., 2015; Zhou, Zhu, & Yao, 2018). These bacteria exude low-molecular-weight organic acids (Figure 5), such as gluconic acid and 2-ketogluconic acid that liberate C from mineral-organic complexes via destabilization and dissolution (An & Moe, 2016), similar to the actions of carboxylates released from plants (Zhou, Zhu, & Yao, 2018). It is therefore reasonable to expect that increases in the relative abundance of P-solubilizing bacteria also play an important

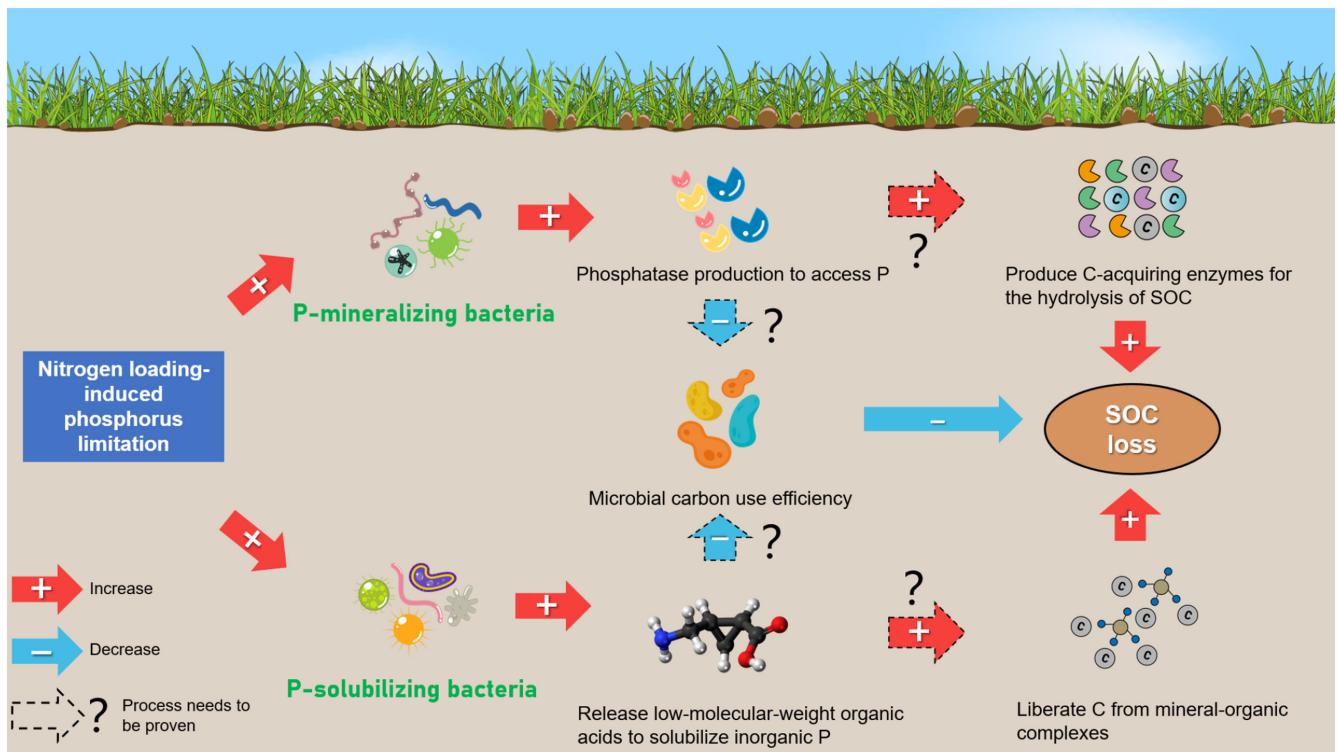


FIGURE 5 Conceptual diagram of microbial phosphorus-acquisition strategies and their impacts on soil carbon (C) cycling under nitrogen loading. Nitrogen-induced phosphorus (P) limitation will increase the relative abundance of P-mineralizing and P-solubilizing bacteria. Phosphorus-mineralizing bacteria will increase production of phosphatase enzymes to access organic P while simultaneously producing C-acquiring enzymes, stimulating soil organic carbon (SOC) decomposition. Phosphorus-solubilizing bacteria will release low-molecular-weight organic acids to mobilize P that also may liberate C from mineral-organic complexes via destabilization and dissolution. Microbial carbon use efficiency (CUE) will decrease under N loading because of C and energy being allocated to the synthesis of enzymes and low-molecular-weight organic acids instead of biomass. A decline in microbial CUE is consistent with reduced production of microbial necromass, leading to a reduction in SOC sequestration and increase in SOC loss.

role in SOC decomposition (Ding et al., 2021), although there is currently few field data to support this hypothesis. Future studies should compare the C-degrading ability of P-solubilizing and non-P-solubilizing bacteria to more clearly quantify the role of P-solubilizing bacteria in soil C decomposition.

4.3 | Microbial carbon use efficiency and its role in soil carbon sequestration

Under N loading-induced P limitation, soil micro-organisms invest energy into the production of enzymes and low-molecular-weight organic acids (Domeignoz-Horta et al., 2020). Therefore, microbial carbon use efficiency (CUE), defined as the fraction of C consumed by micro-organisms that is used for biomass growth (Allison et al., 2010), would decrease with an increase in other investments (Figure 5). The decline in microbial CUE under N loading is consistent with observations that N addition decreases the fungi/bacteria ratios in P-deficient soils (Chen, Li, et al., 2018; Högberg et al., 2007) because bacteria-dominated microbial communities often have a lower CUE (Högberg et al., 2007). Lower microbial CUE can reduce necromass, which contributes up to ~80% of SOC (Fan et al., 2020;

Luo et al., 2020). Therefore, N loading-induced P limitation may decrease SOC sequestration by reducing microbial CUE.

5 | RESEARCH CHALLENGES, PRIORITIES AND IMPLICATIONS

5.1 | Trade-offs between plant phosphorus-acquisition strategies and soil carbon cycling

Plants may adopt multiple P-acquisition strategies under N loading, and each strategy imposes C costs on the plants (Jiang et al., 2019; Lambers et al., 2015). Indeed, mycorrhizal fungi and carboxylate exudation could cost 25%–50% of below-ground C allocation (Lugli et al., 2020). Therefore, plants will likely show trade-offs between strategies used to alleviate P limitation induced by N loading (Ding et al., 2021; Lynch & Brown, 2008). For instance, a study across 14 coexisting subtropical tree species found that species with lower colonization by arbuscular mycorrhizal fungi had higher root branching, whereas species with higher colonization by arbuscular mycorrhizal fungi had consistently lower root branching (Liu et al., 2015). Another study on 10 *Kennedia* species reported that carboxylate

exudation decreased if plants were inoculated with arbuscular mycorrhizal fungi (Ryan et al., 2012). Given these findings, an interesting and open question is how do plants coordinate diverse P-acquisition strategies to respond to N loading? Because P-acquisition strategies can have different impacts on soil C cycling, trade-offs between plant strategies may alter the magnitude or even the direction of the response in soil C storage to N loading. In fact, plant P-acquisition strategies probably interactively respond to their effects on soil C cycling. In future studies, trade-offs between plant P-acquisition strategies under N loading should be investigated to better understand relationships between soil C, N and P dynamics.

5.2 | Ecosystem threshold for phosphorus adaptation

Although plants and soil micro-organisms can adopt a series of strategies in response to low-P conditions, these strategies may be quantitatively insufficient to deliver enough P to maintain the current system state under prolonged N loading (Chen, Xiao, et al., 2020). That is because cumulative N input has the potential to cause N saturation and induce deleterious effects on plant and microbial growth over long-term N loading (Chen, Van Groenigen, et al., 2020). Thus, there may be an ecosystem threshold for P adaption (Jiang et al., 2019), below which plants and soil micro-organisms can maximize short-term P uptake in response to P demand (Figure 6). Beyond this threshold, plants and soil micro-organisms may generally reduce C allocation for P-acquisition, such as decreasing enzyme production, root exudation or fine root biomass (Carrara et al., 2018). In this situation, plant and

microbial P acquisition will not meet P demand beyond the ecosystem threshold, and ecosystem-level P limitations will increase (Figure 6). The dominant plant species may subsequently decrease in biomass, die and/or be replaced by other plant species, which will likely cause other ecosystem-level changes (Figure 6). The new ecosystem's P demand will probably decrease along with having an increased N threshold. Thus, the new ecosystem may be better adapted for increased N loading (Figure 6). Overall, one of the most important challenges in understanding the effect of N loading-induced P limitation on soil C cycling focuses on quantifying an ecosystem threshold of P adaption (Reed et al., 2015). An appropriate time-series analysis of plant and microbial P-acquisition strategies may help determine such a threshold.

5.3 | Native soil nitrogen availability regulates nitrogen loading effects on phosphorus-acquisition strategies

Nitrogen loading induces plants to deploy P-acquisition strategies to cope with P deficiency. However, the role of N loading on P-acquisition strategies also depends on background levels of soil N availability (Chen, Xiao, et al., 2020; Ochoa-Hueso et al., 2011). For instance, in an alpine forest, mycelial biomass and production increased under N addition with relatively low native soil N availability but decreased when native soil N was relatively high (Guo et al., 2021). This is consistent with the idea that when N loading exceeds an ecosystem threshold, N saturation will inhibit plant and microbial P-acquisition strategies (Jiang et al., 2019). However, the effect of N loading on phosphatase activity was

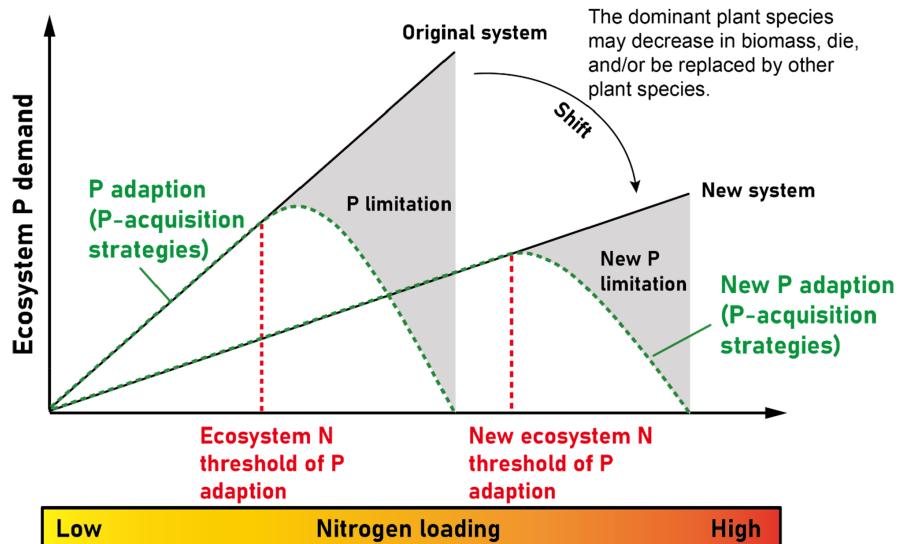


FIGURE 6 Ecosystem threshold for phosphorous (P) adaption under nitrogen (N) loading. When N loading increases, the demand for P (black solid line) will increase. Below the ecosystem threshold (red dotted line), plants and soil micro-organisms can stimulate P-acquisition strategies (green dotted line) to meet the demand for P (black solid line). Beyond the ecosystem threshold, N saturation occurs and will reduce the energy allocated to P-acquisition strategies and increase ecosystem-level P limitation (grey area). When P limitations exceed acquisition capacities, the dominant plant species will decrease their plant biomass, die and/or be replaced by other plant species. Regardless, the new ecosystem P demand will likely decrease while the ecosystem N threshold increases. In this way, the ecosystem can progressively adapt to increased N loading.

significantly weaker at low soil N content than in an ecosystem with high soil N content (Chen, Xiao, et al., 2020). This may be because plant growth was limited in low-N soils even with increased N loading (Fatemi et al., 2016; Jones et al., 2013). Under this condition, N loading will primarily support plant and microbial growth rather than phosphatase production that imposes high N costs (Pii et al., 2015). Nonetheless, these studies demonstrate that native soil N availability affects the response of P-acquisition strategies to N addition. Quantifying the response of these strategies to N loading at different native soil N availabilities is important to understand different P-acquisition strategies across sites and ecosystems.

5.4 | Phosphorous limitations in modelling carbon cycling under nitrogen loading

Given the importance of P for plant and microbial growth, the inclusion of P cycling in Earth System Models (ESMs) could significantly improve our ability to predict future C stocks and fluxes (Wieder et al., 2015). Phosphorus modules have been built into ESMs such as the Carnegie-Ames-Stanford Approach (Wang et al., 2010); Community Land Model-Carbon, Nitrogen, and Phosphorus (Yang et al., 2014); Coupled Model Intercomparison Project-Carbon, Nitrogen, and Phosphorus (Wieder et al., 2015); and Jena Scheme for Biosphere–Atmosphere Coupling in Hamburg (Goll et al., 2012), to investigate C–N–P interactions. Four aspects of P cycling have been included in ESMs: mineralization, sorption, limitation and stoichiometric relationships with C and N (Reed et al., 2015). However, none of these ESMs explicitly consider the existing variety of plant and microbial P-acquisition strategies or their responses to N loading, which increases uncertainties in predicting the effect of ecosystem-level P limitation on soil C fluxes (Chen, Van Groenigen, et al., 2020; Jiang et al., 2019). For instance, some models suggest that future P limitation of plant growth will turn ecosystems into net CO₂ sources by the end of the 21st century (Wieder et al., 2015). However, when plant P-acquisition strategies are considered, ecosystems could persist as net CO₂ sinks with long-term N deposition (Fleischer et al., 2019). Consequently, more realistic ecosystem-level P limitations should be included in modelling soil C cycling to accurately estimate C stocks and fluxes under N loading.

6 | SUMMARY AND CONCLUSION

Plants and soil micro-organisms develop a range of P-acquisition strategies under N loading-induced P limitation, and these strategies can directly and indirectly affect soil C cycling. The release of carboxylate exudates and phosphatase by plants can play an important role in SOC decomposition, whereas symbiotic associations with mycorrhizal fungi and modifications of root

morphological traits can have impacts on SOC stabilization. Increasing the relative abundance of P-mineralizing bacteria may enhance the production of C-acquiring enzymes with significant effects on soil C decomposition, because P-solubilizing bacteria produce low-molecular-weight organic acids, which can mobilize mineral-associated C in soils. As more resource is allocated into microbial enzyme and low-molecular-weight organic acid production, microbial CUE will decrease, which, in turn, can reduce SOC sequestration.

Future research is required to explore the relationships between plant and microbial P-acquisition strategies and soil C cycling, such as clarifying trade-offs between strategies, determining the ecosystem threshold of P adaptation and understanding how soil N availability regulates the development of P-acquisition strategies. We envision that including different P-acquisition strategies can significantly improve model projections of soil C cycling.

AUTHOR CONTRIBUTIONS

Ji Chen and Min Luo designed the study. Min Luo, Daryl L. Moorhead, Raúl Ochoa-Hueso, Carsten W. Mueller, Samantha C. Ying and Ji Chen wrote the manuscript. All authors contributed substantially to the revisions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Ji Chen is an Associate Editor of Functional Ecology, but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

There are no data to archive.

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