How do terrestrial plants access high molecular mass organic nitrogen, and why does it matter for soil organic matter stabilization?

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Abstract Although there is increasing awareness of the potential role of organic N compounds (ON) in plant nutrition, its implications for soil organic matter (SOM) stabilization have hardly been discussed yet. The aim of this paper is therefore to gather the newest insights into plant use of high molecular mass organic N, its effect on root growth and anatomy, and finally, to discuss the implications of plant use of organic N for SOM stabilization. I propose that modified root growth due to the uptake of ON provides greater root and root-associated microbe input, leading to enhanced SOM stabilization. Finally, I discuss the role of the proposed framework in different ecosystems, and I encourage future studies combining plant N nutrition and SOM stabilization.

Keywords N acquisition · N cycle · Soil decomposition · Soil proteolysis

Introduction

Nitrogen (N) is a major nutrient determining plant productivity and thus it exerts strong control on soil organic matter (SOM) formation as plant litter

provides carbon (C) and N-rich input (Vitousek and Howarth 1991; Knicker 2011; Kicklighter et al. 2019). However, fossil fuel combustion and the intensive use of synthetic fertilizers have deteriorated the N cycle (Mulvaney et al. 2009; Suddick et al. 2012). The interlinked C cycle has therefore also been impaired, resulting in a decrease of C sequestration (Fung et al. 2005). Thus, solutions that counteract further deterioration of these cycles with a concomitant increase of SOM stabilization are urgently needed.

SOM is a mixture of mostly labile organic biomolecules derived from litter degradation forming supramolecular aggregates stabilized by biochemical recalcitrance and interactions, mineral adsorption, and physical inaccessibility (Simpson et al. 2002; Schmidt et al. 2011; Clarholm et al. 2015; Adamczyk et al. 2019b). The SOM nitrogen pool is mainly composed of proteins/peptides with amino sugars as a second important fraction (Schulten and Schnitzer 1997; Knicker 2011). Although the traditional paradigm includes only the soil inorganic N (IN) pool as directly available to plants, more recent studies point to the direct acquisition of organic N (ON) compounds, i.e. urea (Mérigout et al. 2008), quaternary ammonium compounds (Warren 2013), amino acids (Näsholm et al. 2009) and short peptides (Tegeder and Rentsch 2010; Soper et al. 2011). Corresponding transporters for these N forms have also been recognized (Breitkreuz et al. 1999; Williams and Miller 2001; Svennerstam et al. 2008; Paungfoo-Lonhienne et al. 2009; Tegeder and Rentsch 2010;

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Perchlik et al. 2014; Ganeteg et al. 2017). Moreover, it has been suggested that plant roots can directly take up proteins (Paungfoo-Lonhienne et al. 2008), DNA (Paungfoo-Lonhienne et al. 2010a) or even whole microorganisms (Paungfoo-Lonhienne et al. 2008, 2010b; White et al. 2015, 2018). In addition, roots directly enhance soil proteolysis with root-derived proteases (Godlewski and Adamczyk 2007; Paungfoo-Lonhienne et al. 2008; Adamczyk et al. 2010b) and indirectly through rhizosphere priming (Cheng et al. 2013) (Fig. 1).

Taking up ON does not leave root growth and plant biomass allocation unaffected (Cambui et al. 2011). The uptake of ON potentially leads to higher root growth compared with IN (Paungfoo-Lonhienne et al. 2009; Rasmussen et al. 2014; Lonhienne et al. 2014a). Similarly, rhizosphere microbial biomass increases under ON fertilization compared to IN (Ikoyi et al. 2020; Tang et al. 2020; Peltoniemi et al. 2021). Given that roots and root-associated microbes are the main source of stable SOM (Clemmensen et al. 2013), enhanced root and microbial growth may increase SOM stabilization. It has recently been shown that plant roots increase soil ON pool, and interaction between root-derived compounds with fungal necromass leads to SOM stabilization (Adamczyk

et al. 2019a, b). Hence, increased root growth could enhance the soil ON pool and SOM stabilization.

In this opinion paper, I aim to gather the newest information on the plant use of high molecular mass ON and its effect on root growth, anatomy, and SOM stabilization. I hypothesize that increased root growth due to the uptake of ON provides higher root and root-associated microbe inputs, leading to enhanced SOM stabilization. The paper is divided into three parts. The first gathers the newest insights into plant use of high molecular mass ON (via direct uptake and after depolymerization with root-derived proteases). The second provides information on how ON fertilizers affect root biomass and architecture. The third links the use of ON with SOM stabilization. Finally, I briefly analyze the impact of the proposed framework for different ecosystems and identify future directions.

Plant mechanisms to access high molecular mass N sources

There is now mounting evidence that ON can be directly used by plants. Groundbreaking work on the use of intact amino acids has been published (Näsholm et al. 2009), and uptake of quaternary ammonium compounds was demonstrated (Warren 2013). Moreover, more than one hundred organic N compounds were found to be exuded and taken up by wheat roots, including not only amino acids, but also polyamines, nucleobases and nucleosides (Warren 2015). The release of ON from the SOM supramolecular structure has recently been intensively studied (Clarholm et al. 2015; Keiluweit et al. 2015; Keiluweit and Kuyper 2020; Wang et al. 2020). Thus, here I will concentrate on plant access to SOM-released high molecular mass ON via indirect and direct pathways.

Direct uptake of proteins and microorganisms

Although the direct uptake of whole intact proteins by *Allium cepa*, *Hordeum vulgare*, *Solanum lycopersicum* and *Zea mays* roots was already mentioned decades ago (Seear et al. 1968), this phenomenon has been waiting for long time to be proposed again. A study with *Arabidopsis thaliana* showed that roots take up intact proteins potentially via endocytosis

Fig. 1 Plant mechanisms for accessing soil nitrogen (N) pools. AAs amino acids

(Paungfoo-Lonhienne et al. 2008). However, according to another study, minor damage of roots is necessary for protein uptake by *Triticum aestivum* (Rasmussen et al. 2015). In addition to whole proteins, it has been suggested that *A. thaliana* roots take up whole bacteria and yeast cells. As after uptake these microbes are degraded in root cells, this phenomenon has been called “rhizophagy” (Paungfoo-Lonhienne et al. 2010b). A further crucial step has been taken by White and co-workers, who proposed the term “rhizophagy cycle” and proved that it is widespread for vascular plants (White et al. 2018). In the rhizophagy cycle, microbes alternate between a root endophytic phase and a free-living soil phase, providing N for the plants (White et al. 2018). Studies on *T. aestivum* have suggested that the importance of soil microbe uptake to N cycling is relatively minor compared with fluxes of other forms of IN and ON (Hill et al. 2013). However, the available knowledge on the uptake of whole proteins and microorganisms is very limited. This means that an estimation of the role of such a process and its commonness in the plant kingdom remains impossible. The ability to take up whole proteins and microorganisms would constitute a potentially very efficient mechanism to access soil protein N. Uptake of whole proteins could be increased under specific circumstances, like intensive ON fertilization and drying-rewetting events providing dead microbes and soil animals in the rhizosphere.

Indirect plant mechanisms to access protein N

According to the traditional point of view, soil proteases originate only from free-living and plant-associated microbes. Trees form symbioses with ectomycorrhizal fungi (ECM), very efficient degraders of SOM able to secrete proteases (Smith et al. 2015; Brundrett and Tedersoo 2018). However, the vast majority of vascular plants, including agricultural species, form symbioses with arbuscular mycorrhizal fungi (AMF), which do not enhance soil proteolysis like ECM. Yet, AMF enhance plant ON acquisition via the ability to take up amino acids (Whiteside et al. 2012) and by increasing the volume of exploited soil (Lambers et al. 2019). Moreover, roots per se increase proteolysis (and thus, access to protein N) via two routes. Roots enhance soil protein depolymerization by supporting rhizosphere microorganisms with

easily available C sources, a phenomenon known as “rhizosphere priming effect” (Kuzakov et al. 2000; Dijkstra et al. 2013; Cheng et al. 2013). Numerous plant species also enhance proteolysis directly via root-derived proteases, which include enzymes associated with the root surface (Chang and Bandurski 1964; Greenfield et al. 2020) and actively secreted by the roots (Godlewski and Adamczyk 2007) or root border cells (Brigham et al. 1995; Hawes et al. 2000, 2016; Miyasaka and Hawes 2001; Pan et al. 2004; Wen et al. 2007). Although root surface-associated enzymes are not secreted from roots, they may be even more important for soil decomposition than secreted ones. This is due to the spatial factor; increased proteolysis in the vicinity of the root potentially decreases competition with microorganisms for released ON, prioritizing plant N uptake.

The knowledge of root-derived proteases and control over their activity is scarce, making yet the assessment of their role in the plant N budget challenging. An interesting approach would therefore be to compare mechanisms controlling the activity of root-derived proteases with those of root-derived acid phosphatases, as they share crucial similarities. For both enzymes, acid phosphatases and proteases derived from numerous crop species, the presence of organic forms of N or P in the culture media enhanced their activities (Yadav and Tarafdar 2001; Tarafdar and Claassen 2003; Adamczyk et al. 2008, 2010a), and the highest plant growth was obtained on media with inorganic and organic forms of P (Tarafdar and Claassen 2003) and N (Paungfoo-Lonhienne et al. 2008; Adamczyk and Godlewski 2010).

The greatest challenge here is to extrapolate laboratory axenic experiments to heterogeneous nonsterile conditions and assess the role of the abovementioned phenomena in plant N nutrition. The first attempts have already been made, and they show that the contribution of organic N to total N uptake by grassland plants (*Kobresia humilis*, *Saussurea superba* and *Stipa aliena*) at alpine meadows ranged between 13 and 35% (Xu et al. 2006). Similarly, newly depolymerized high molecular ON contributed to the N nutrition of *Z. mays*, accounting for 20 to 30% of N uptake (Enggrob et al. 2019). The role of root-derived proteases from *Z. mays* and *T. aestivum* for soil proteolysis has also been assessed to be up to 20% of total soil proteolytic activity (Greenfield et al. 2020).

The origin of the enzymes (microbes vs plant roots) could be identified with metaproteomics methods (Bastida et al. 2018). Future studies should consider heterogeneous ON sources, including recalcitrant soil ON pools (Adamczyk et al. 2019a, b) and hotspots of high molecular mass ON. In addition, spatial and temporal differences in N availability due to factors like drying-rewetting cycles (Warren 2013) or dead soil animals close to the roots should be taken into account. Moreover, as high molecular mass ON depolymerization is driven mainly by substrate availability and is not an enzyme-limited process this should also be assessed in future studies concerning plant ON use (Noll et al. 2019; Hu et al. 2020).

Effect of different ON forms on root anatomy

While ON uptake by plants is an increasingly recognized phenomenon, its effect on root growth, functioning, and anatomy is far less known. According to the current state of knowledge, plants growing on medium with ON allocate more biomass to the roots than those growing on IN; in addition they retain most of the ON taken up in the roots. This effect has been observed for low molecular mass ON (amino acids) (Cambui et al. 2011), but also for high molecular mass ON uptake by *A. thaliana*, *T. aestivum*, and *Hakea actites* (Paungfoo-Lonhienne et al. 2008; Rasmussen et al. 2014; Lonhienne et al. 2014a). The form of N taken up and plant N status affect not only root biomass, but also the root architecture, in a similar manner as P deficiency and the presence of organic P (OP). In P-deficient conditions, some plant species form “root clusters” including proteoid roots (in species from the Proteaceae and Fabaceae families, and many others) and dauciform roots (in some Cyperaceae species) (Shane and Lambers 2005; Nge et al. 2020). These roots secrete acid phosphatases in an “exudative burst” to decompose OP and exude carboxylates to suppress the adsorption of released Pi to mineral surfaces (Lambers et al. 2006, 2013; Playsted et al. 2006; Wang and Lambers 2020). Similarly, cluster roots are formed in response to N-deficiency, but also in the presence of high molecular mass ON (Paungfoo-Lonhienne et al. 2009). The enrichment of the culture media with protein increased not only the formation of cluster roots of *H. actites* but also enhanced the expression of a

putative peptide transporter, indicating the important role of cluster roots in ON uptake (Paungfoo-Lonhienne et al. 2009). Changes in root anatomy in the presence of proteins in the culture medium were also observed for plant species which do not form cluster roots. Protein in the culture medium turned the wheat (*T. aestivum*) root structure into a knob-like one (Rasmussen et al. 2014). The enhancement of root growth compared to IN addition was observed only for lower protein concentrations, while high protein concentrations inhibited root growth of *T. aestivum*, *H. actites* and *A. thaliana* (Paungfoo-Lonhienne et al. 2009; Rasmussen et al. 2014; Lonhienne et al. 2014a). Similarly, short peptides and amino acids in lower concentrations exert a positive effect on root growth compared to IN, and high amino acid concentrations lead to complete growth inhibition of *Lobelia anceps* and *A. thaliana* (Soper et al. 2011).

A logical assumption could be that increased root growth due to the uptake of ON decreases plant yield compared to IN fertilization. However, *A. thaliana* plants benefit from ON uptake in many ways, including an increase of resistance to root pathogens (Lonhienne et al. 2014b) and *Glycine max* increased absorption of water and nutrients (Teixeira et al. 2018). Available knowledge synthesized here therefore suggests that we can shape root growth and architecture via the proper management of ON fertilization.

Linking access to high molecular mass ON with root anatomy and SOM stabilization

Combining the ability of plants to use ON with its effect on root growth and architecture brings a new avenue for understanding root-driven SOM changes. Here, I propose a framework for integrating plant ON nutrition with SOM stabilization (Fig. 2). Increased root growth due to the uptake of ON leads to higher root and root-associated microbe inputs to the soil. Given that roots and associated microbes build up most of the stable SOM (Clemmensen et al. 2013; Liang et al. 2017), increased input from these sources should result in an increase of SOM build-up. However, a limitation with this approach is that root-derived inputs influence not only SOM stabilization, but also its formation and turnover (Clemmensen et al. 2013; Bastida et al. 2019; Sokol and Bradford 2019). The recently introduced framework of Rhizo-Engine

Fig. 2 Framework combining plant nitrogen (inorganic vs organic) uptake with SOM decomposition and stabilization. On the pink and green background effect of IN vs ON fertilization (+IN vs +ON) are depicted. The balance between SOM

decomposition vs stabilization is affected also by factors presented as weights (in grey background). *IN* inorganic nitrogen, *ON* organic nitrogen, *SOM* soil organic matter, *soil chem.* soil chemistry

(Dijkstra et al. 2020) proposed four potential scenarios for root-driven soil organic C (SOC) dynamics. These include a) a destabilizing scenario, in which roots accelerate the formation of unprotected SOC and its decomposition, and b) a stabilizing scenario, in which roots accelerate the formation of protected SOC and accelerate the decomposition of unprotected SOC. Thus, how can we predict the effect of increased root growth due to plant ON uptake on SOM stabilization vs destabilization? The root-driven effect on SOM changes seems to be affected by the type of ecosystem and land use, N availability, climate conditions, disturbances, plant species, rhizosphere microorganisms, and soil chemistry (Fig. 2). Below, I will briefly discuss the implications of these factors for the framework proposed in this paper.

In low N availability ecosystems (e.g. boreal forests) with a dominant soil ON pool, plants partition more biomass to the roots (Chapin 1980; Levin et al. 1989), and ON uptake by plants and microbes provide a C surplus (Franklin et al. 2017). Thus, in line with the framework proposed in this paper ON uptake leads to SOM stabilization. In contrast, in high N availability ecosystems (e.g. agriculture with heavy IN fertilization), more biomass is partitioned to the shoots, the soil N pool is IN-richer, and there is no C surplus during N uptake (Clarholm 1997), SOM is therefore not/less stabilized. Thus, in high N availability ecosystems, ON fertilization could turn the soil N pool into a more ON orientated one with enhanced root growth and SOM build-up. In support of this, a long-term fertilization study of a *H. vulgare-G.*

max crop rotation showed that ON fertilization with manure increased SOC sequestration compared with IN fertilization (Guo et al. 2019).

SOM stabilization is affected by plant-soil-microbe interactions including plant and microorganism biodiversity. Plants' preferences in taking up ON are species-specific (Liu et al. 2017), similarly as is the activity of root-derived proteases (Adamczyk and Godlewski 2010) and plant root-chemistry. All these factors may influence the effect on SOM stabilization (Coq et al. 2011; Sun et al. 2018; Adamczyk et al. 2019a; Hättenschwiler et al. 2019). Rhizosphere microorganisms affect SOM stabilization, and roots with mycorrhizal fungi are the source of the most stable soil C (Clemmensen et al. 2013). However, some ECM fungi, e.g. *Cortinarius acutus*, restrict SOM accumulation (Clemmensen et al. 2015; Kyaschenko et al. 2017; Lindahl et al. 2021), which underlines the need to understand the microbial community structure. Soil chemistry also affects SOM stabilization, e.g. soils rich in minerals bind more organic matter (Mikutta et al. 2006; Schmidt et al. 2011).

Climate change brings high temperatures, changes in precipitation, and N deposition, which adds more N to the ecosystem. The available experiments aiming to disentangle the effect of N deposition on C stabilization in forest ecosystems with low N availability mainly show an increase of C sequestration. The reason could be decelerated decomposition (Hyvönen et al. 2008; Janssens et al. 2010; Liu and Greaver 2010) or changes in SOM chemistry, i.e. more lignin derivatives and microbial N compounds

(Hasegawa et al. 2021). Alternatively, such effect was explained by an increase of plant productivity leading to increased litter input (Forsmark et al. 2020). Atmospherically deposited IN in forest ecosystems with low N availability is rapidly immobilized (Sheng et al. 2014) by microbes, potentially providing ON-rich microbial necromass for plants, mimicking ON fertilization in ecosystems with high N availability. However, in arable soils which are not so N-limited as forests (Sawada et al. 2015) meta-analysis showed that N deposition decreased microbial biomass and enhanced N leaching (Treseder 2008; Verma and Sagar 2020). Thus, the effect of N deposition depends on N availability of ecosystems.

Plants are thus able to access a wide set of N forms via multiple mechanisms which affects SOM stabilization. However, why do plants access ON? Why do they not wait for microbes to decompose ON to IN? First, the dominant soil N pool contains high molecular mass ON compounds (Clarholm et al. 2015; Knicker 2011), so the ability of plants to directly access this ON pool would be beneficial in N limiting ecosystems. Second, depolymerization is the crucial step in making high molecular mass ON available (Schimel and Bennett 2004; Jan et al. 2009), peptides and amino acids dominate as the products of enzymatic depolymerization of soil organic N (Warren 2021). As these peptides are taken up more quickly from soil than equivalent N monomers (amino acids) (Farrell et al. 2013), the plant's ability to compete for these N forms with soil microbes already at this stage is highly advantageous. Third, the carbon bonus of organic N uptake increases the plant N use efficiency (Franklin et al. 2017).

Conclusions

On the basis of the laboratory studies undertaken to date, we know that plants directly take up not only IN, but also ON, including amino acids, proteins, or even whole microorganisms. Moreover, plants directly increase soil proteolysis via root-derived proteases (root-surface associated or secreted from roots), and indirectly via rhizosphere priming.

Here, I propose that high molecular mass ON use increases root growth and turns the soil N pools to a more ON-oriented pathway, which results in enhanced SOM stabilization. The use of high molecular mass

ON by plants opens new avenues for climate-smart organic N fertilization, with concomitant SOM stabilization. However, the direction of root-driven SOM changes needs more study, taking into account plant species, rhizosphere microorganisms, and the soil physicochemistry. Given all the biological advantages of accessing high molecular mass ON and the need to decrease the use of IN fertilizers for mitigating climate change, work on this subject needs to continue, despite many challenges.

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Declarations

Conflicts of interest/competing interests Not applicable

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