Organic nitrogen storage in mineral soil: implications for policy and management

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Abstract

Nitrogen is one of the most important ecosystem nutrients and often its availability limits net primary production as well as stabilization of soil organic matter. The long-term storage of nitrogen-containing organic matter in soils was classically attributed to chemical complexity of plant and microbial residues that retarded microbial degradation. Recent advances have revised this framework, with the understanding that persistent soil organic matter consists largely of chemically labile, microbially processed organic compounds. Chemical bonding to minerals and physical protection in aggregates are more important to long-term (i.e., centuries to millennia) preservation of these organic compounds that contain the bulk of soil nitrogen rather than molecular complexity, with the exception of nitrogen in pyrogenic organic matter. This review examines the factors and mechanisms that influence the long-term sequestration of organic nitrogen in mineral soils. It examines the policy and management implications which stem from this newly accepted paradigm, such as critical loads considerations and nitrogen saturation and mitigation consequences. Finally, it emphasizes how essential it is for this important but underappreciated pool to be better quantified and incorporated into policy and management decisions.

1 Introduction

Nitrogen (N) is an essential nutrient which limits productivity in most terrestrial ecosystems (LeBauer and Treseder, 2008; Chapin III et al., 2012). Anthropogenic activity has doubled the rate of terrestrial N fixation and has caused many regions to experience significant impacts from N enrichment such as eutrophication, acidification and loss of biodiversity (Canfield et al., 2010). Soil is the largest pool of fixed and biologically available N; decades of research have improved our understanding of the pathways N moves through in the soil environment, the nature of soil organic N (SON) and its implication for C sequestration, work that has been synthetized in important reviews (van...
Because most anthropogenic N additions end up in the soil (Johnson and Turner, 2014), a thorough understanding of the processes and factors governing its removal from cycling and sequestration is crucial for informing policy and management decisions, yet this information remains scattered throughout the literature.

Despite the fact that mineral N fixed on clays can make a significant contribution to sub-surface soils N stocks (Stevenson, 1986), the bulk of the soil N stock is in organic matter forms (Schulten and Schnitzer, 1998). Analytical advances in the past few decades have altered our understanding of soil organic matter (SOM) chemistry and resulted in a paradigm shift of the mechanisms explaining its long-term persistence (M. W. I. Schmidt et al., 2011). For many years it was believed that chemical recalcitrance, i.e., the inherent resistance to degradation, was responsible for the longevity of organic matter in soils. Classically, two mechanisms were thought to foster SOM persistence: (1) selective preservation of structurally complex compounds, such as lignin, suberin and cutin, due to their resistance to microbial breakdown, and (2) condensation reactions forming intricate, irregular organic compounds generally referred to as humus (Kögel-Knaber, 1993; Stevenson, 1994; Sollins et al., 1996). However, empirical evidence of humification has never been found, and advanced chemical analyses of the persistent organic matter pool in soil demonstrated that it is primarily made of chemically labile structures with low relative abundance of aromatic groups (Kleber et al., 2011, and references therein). Thus, currently the persistence of organic matter in soil is believed to be an ecosystem property (M. W. I. Schmidt et al., 2011), controlled by microbial inhibition, physical protection and/or chemical stabilization (Von Lützow et al., 2006).

Many excellent reviews are available outlining the changed understanding of the persistence of SOM (Von Lützow et al., 2006; Sollins et al., 2007; Kögel-Knabner et al., 2008; Marschner et al., 2008; Nannipieri and Paul, 2009; Deb and Shukla, 2011). To date though, there has not been a comprehensive examination of the implications of this new SOM stabilization paradigm for long-term N sequestration in naturally func-
tioning ecosystems. Because of the importance of N sequestration to ecosystem functioning, as well as to policy considerations such as critical loads, an examination of the factors controlling N sequestration within this revised SOM paradigm is crucial. In this review we will first synthesize the current understanding of the nature of the soil N pool and of the factors controlling its long-term storage. We will then examine the factors governing the currently understood mechanisms of N persistence: microbial processing, chemical protection and physical protection from microbial degraders (Fig. 1). Finally, we will examine how recent changes in paradigm may affect policy and management actions undertaken to protect and remediate natural areas experiencing increased N deposition, and elucidate why land managers should care about this new understanding.

2 Nitrogen in soils

Nitrogen exists in both inorganic (primarily as ammonium \([\text{NH}_4^+]\) and nitrate \([\text{NO}_3^-]\)) and organic forms within the soil. Mineral N is introduced to the soil as a product of microbial fixation or through atmospheric deposition, primarily as the result of anthropogenic fossil fuel combustion and emissions from agricultural practices, especially confined animal feedlots, though N inputs from rock weathering may be present as well (Thamdrup, 2012; Houlton and Morford, 2015). Yet, it has long being recognized that most often, the primary form of N in soil is organic, with Jodidi (1911) noting over a century ago that “practically all of the N occurring in soils is of an organic nature”. In fact, organic compounds can comprise up to 95% of the N in some soils (Rillig et al., 2007; Nannipieri and Paul, 2009; Knicker, 2011), with amides and amines making up the majority of the organic N pool, and aromatic N compounds, while present, contributing a relatively minor share (Leinweber et al., 2013). Most of this organic N enters the soil as particulate organic matter (POM) through above-ground plant litter and root litter, or in the form of pyrogenic organic matter after fire (Knicker, 2011). Dissolved organic nitrogen (DON) enters the mineral soil through root exudates and leaching of soluble components of
plant litter in the organic (O) horizon (Knicker, 2011; Qualls and Haines, 1991) and concentrations and fluxes of DON entering the mineral soil from the O horizon generally decrease quickly with depth (Rosenqvist, 2010). Measurements at the Hubbard Brook Experimental Forest in New Hampshire, a California mudflow chronosequence and European sites have found that in some systems, especially those with leaf litter having a higher %N, above-ground inputs can be an important source of DON, providing from half to more than double the input of N to the mineral soil compared to below-ground inputs (Dittman et al., 2007; Sleutel et al., 2009; Uselman et al., 2012). The amount of DON in litter leachates varies linearly with the litter N concentration (Soong et al., 2015). In the field, leachate from pine litter was observed to have less DON than that from oak or mixed litter (Silveira et al., 2011). Sleutel et al. (2009) observed that DON comprised 28–46 % of dissolved N-fluxes into the mineral soil in deciduous forests in high N deposition environments, compared with less than 20 % as DON in coniferous forests, and these patterns have been noted in other studies (Currie et al., 1996). Nitrogen is also introduced to the soil through root litter and exudates. In some cases, these inputs may be as or more important than leaf litter inputs for DON (Uselman et al., 2009; B. H. M. Schmidt et., al., 2011; Uselman et al., 2012). Root N introduced to mineral soil layers is likely to already be in a form that is able to be rapidly processed by microbes and transformed into low molecular weight forms of N (Sanaullah et al., 2011).

In fire affected ecosystems, organic N may also enter the soil in the form of pyrogenic organic nitrogen (Py N). Py N is believed to contribute largely to the heterocyclic N forms found in soils and to be the only SON pool preserved for long time spans by inherent recalcitrance (Knicker, 2011). In fact, Py N appears to be locked away from the internal N cycling, causing N limitation in frequently burned sites (Soong and Cotrufo, 2015).
2.1 Microbial processing

Nitrogen that is stored for centuries to millennia is predominantly composed of low molecular weight, labile molecules. These compounds can derive directly from plants, as in the case of simple sugars and amino acids, or they may be derived secondarily from microbial processing such as exudation or cell death (Rillig et al., 2007, and references therein). Highly labile compounds such as free amino acids do not exist for long in the soil and are taken up rapidly, within hours or even concurrent with production in some cases (Farrell et al., 2011); because nearly all N sequestered for long periods has undergone microbial processing (possibly many times over) before it is removed from circulation, microorganisms exert an important influence in regulating the amount of N suitable for long term storage (Sollins et al., 2007; Knicker, 2011). Microbial community composition, as well as environmental factors such as soil pH, moisture and temperature exert important influences on microbial activity and the breakdown of proteins within soils (Sylvia et al., 2005).

The proportion of N in substrate is often used as a proxy for its quality and lability. The N content of SOM has long been recognized as an important control over decomposition (Grandy et al., 2009). Observations that leaf litter with a higher %N also had a higher DON concentration (Uselman et al., 2012; Soong et al., 2015) suggests that a higher amount of N suitable for storage may be produced from higher quality OM. Thomas et al. (2009) used soils from a wide range of biomes to find that the quality of substrate inputs is a main driver of SOM composition. In a crop amendment study, St. Luce et al. (2014) found greater incorporation of N into microbial biomass and the mineral N pool with low C:N faba bean residue than with high C:N wheat residue additions. Gillespie et al. (2014) expanded this view from plant residue additions to any type of N inputs as they observed an increase of N-containing microbial byproducts incorporated into the fine fraction following fertilization with mineral N or manure.

High throughput of these higher quality materials can result in a greater stabilization of SOM, resulting in what would have been earlier considered a paradox: that
greater amounts of labile material result in greater amounts of N retained in the soil; a pattern noted by Giardina et al. (2001) in a litter comparison experiment. Cotrufo et al. (2013) have proposed a framework whereby increased substrate quality leads to higher amounts of microbially processed organic matter and thus potential for stored N. According to this framework, litter with more labile forms of C as well as higher %N both lead to increased microbial substrate use efficiency, resulting in a greater production of microbial residues. Evidence for this has been found by Hatton et al. (2015) who used isotopic tracers in a comparison of root and needle litter to find that more labile needle litter preferentially accumulated in slow-cycling SOM fractions composed of microbial byproducts than did less labile root material. In litterbag experiments, Hobara et al. (2014) found higher amounts of N in mineral soils and greater amounts of labile, microbial byproducts (amino acids and amino sugars of bacterial origin) from litter with higher initial %N and of higher initial quality.

Other evidence indicates that edaphic factors may be as or more important than litter quality in determining the amount of microbial N processing and their substrate use efficiency in soils (Manzoni et al., 2012; Sinsabaugh et al., 2013). In addition, Kaiser et al. (2014) recently observed that microbial community composition may be able to buffer compositional differences of OM and maintain a constant level of substrate use efficiency despite different C:N ratios and substrate nutrient content. Similarly, in mesocosm experiments, soil characteristics were found to be more important than litter quality for C and N transformation rates (Delgado-Baquerizo et al., 2015). Clearly more research is required to ascertain the effects of substrate chemistry on microbial OM turnover and production of compounds which can contribute to SON storage.

Existing stocks of accessible C and N in soil also affect the processing of substrate by microbes. Nitrogen enrichment acts to suppress degradation and microbial growth (Frey et al., 2014). In soils with abundant N, amino acids are taken up by microbes primarily for their C-content and N is excreted as NH$_4^+$ to the soil following deamination within the cell (Tahovská et al., 2013; Farrell et al., 2014). Although NH$_4^+$ concentrations and mineralization rates may be high, long-term storage is generally suppressed...
as \( \text{NH}_4^+ \) is taken up by plants and microbes or converted to \( \text{NO}_3^- \) that can be lost from the system; not much N is in organic forms suitable for long-term storage. In contrast, N-limited soils with a higher C:N ratio generally favor an organic-N nutrient economy where N exists primarily in organic form and is rapidly assimilated by plants and microbes which compete against one another (Schimel and Bennett, 2004; Nannipieri and Paul, 2009; Geisseler et al., 2010). In these environments, organic compounds such as amino acids and sugars are taken up directly and are utilized as a source for both C as well as N, which is incorporated into cell biomass (Geisseler et al., 2010). Instead of being excreted to the soil as excess \( \text{NH}_4^+ \), N is returned to the soil solution through exudation or cell death and lysis in the form of low molecular weight N compounds and cell wall fragments suitable for long-term storage. As long as C remains abundant relative to N, microbial biomass will be considered a stoichiometric sink for N and creation of low molecular weight compounds suitable for N storage will be enhanced (Kopáček et al., 2013; Tahovská et al., 2013). The amount and chemical composition of C in the soil also influences microbial transformations, providing an energy source for microbial activity and increasing the rate of OM cycling, increasing the amount of low molecular weight materials (Gleixner, 2013). Another factor to consider is that when N is limiting and fresh inputs of labile material are not available, microbes will attack more resistant N sources, transforming more difficult to decompose N compounds into labile microbial byproducts enriched in N compared to the original SOM (Fontaine et al., 2011; Gleixner, 2013). However, due to the heterogeneity of soils, C and N status is likely to vary widely within short distances, making the interplay between C and N quality and quantity difficult to generalize. These factors play prominent roles in determining the fate of N and C in soils and better understanding of these interactions will pay dividends.

### 2.2 Adsorption

Nitrogen-containing compounds can adsorb directly onto mineral surfaces, retarding transport of these molecules within the soil and reducing susceptibility to oxidative at-
tack (by blocking enzyme attachment, Kögel-Knabner et al., 2008). Adsorption capacity and strength can vary across soil environments and landscapes because of different binding mechanisms, and mineral and protein properties. Proteins adsorb to most surfaces over a wide range of pH and this attachment happens very quickly (Sollins et al., 2007; Yu et al., 2013; Scott and Rothstein, 2014). Adsorption can occur through several processes that may occur simultaneously on different parts of the N-containing molecule. Polyvalent cation bridges and especially ligand exchange are considered strong binding mechanisms while weaker interactions such as van der Waals forces or H-bonding can also occur (Sanderman and Maddern, 2014; Von Lützow et al., 2006).

Several factors are important to the adsorption capacity of a soil including mineral and protein properties, the pH of the solution and the presence of antecedent OM (Von Lützow et al., 2006; Yu et al., 2013).

The size and structure of the protein affects adsorption to mineral surfaces. Larger molecules may offer several adsorption sites for binding, enhancing the strength of the connection to the mineral, as well as limiting the number of active sites available to enzymes (Von Lützow et al., 2006). On the other hand, larger proteins tend to be more highly folded and may offer fewer sites for adsorption, and may be too large to fit within the interlamellar areas of clay minerals (Yu et al., 2013). Charge properties of proteins affect the type and strength of bonding, and even whether the protein is attracted or repelled by the mineral surface (Yu et al., 2013).

The presence of clays in soils is important to N storage, though recent research has added nuance to the process by which OM is retained. Phyllosilicate minerals play an important role in OM retention and are often associated with higher levels of N retention (Deb and Shukla, 2011; Grandy et al., 2009). The high amount of surface area of clay particles leads to a larger number of charged sites and a greater number of binding sites for microbial byproducts. The overall negative charge, small pore spaces and expandable nature of some clay minerals can further increase this capacity (Rillig et al., 2007; Von Lützow et al., 2006). Non-expandable minerals such as kaolinite or quartz experience weak bonding to SOM, while expandable layer phyllosilicates such
as montmorillonite feature extensive internal surfaces available for adsorption (Von Lützow et al., 2006; Yu et al., 2013). These small spaces, along with the small pore spaces associated with clay mineral particles facilitates multiple bondings with OM, resulting in stronger retention and enhanced adsorption capacity (Kögel-Knabner et al., 2008). Vogel et al. (2014) were able to discern that OM was not attached uniformly to the exterior of clay particles; edges and rough surfaces acted as nuclei for OM attachment. Alternatively, Wei et al. (2014) found that OM decomposition was higher with increasing clay content, likely due to the higher bacterial biomass facilitated by a larger amount of pore space and increased substrate supply. Recent work by Vogel et al. (2015) has also found the lack of a relationship between surface area and OM accumulation and also implicated higher microbial biomass as an important factor in OM and N sequestration. These conflicting results show that clay content alone may not be useful as a proxy for long-term N storage potential.

The composition of the minerals plays an important role. Poorly crystalline Fe-oxides and Al-silicates exhibit properties which lead to increased adsorption of OM and N, especially in subsoils and at lower pH, and may provide better adsorption capacity than phyllosilicate clays (Kaiser and Zech, 2000; Sleutel et al., 2009; Dippold et al., 2014). In a study of 41 mineral horizons across Canada, Kothawala and Moore (2009) found that the amount of poorly crystalline Fe and Al controlled the adsorption of DON while Dümig et al. (2012) found Fe oxihydroxides dominated the accumulation of OM on mineral surfaces and the weaker cation bonding led clay minerals to play a minor role. As depth increases, the importance of sorption over aggregate formation as a retention mechanism increases, and the presence of Fe and Al-oxides as an important factor may also increase (Muni et al., 2010; Rumpel et al., 2012). Because the abundance of Fe and Al minerals generally increases with depth, strong correlations between Fe-oxide content and OM retention have been observed in subsoils (Kögel-Knabner et al., 2008; Deb and Shukla, 2011).

The pH of the DON solution can be one of the most important factors in adsorption of proteins to minerals by affecting the surface charge of the mineral and the degree of
ionization of the protein (Yu et al., 2013). The prevalence of ligand exchange, a strong bonding mechanism, increases as pH decreases, and in acidic conditions most OM is associated with Fe-oxides and Al-silicates (Von Lützow et al., 2006; Kögel-Knabner et al., 2008). Ligand exchange is not possible on clay particles without Fe or Al-oxide coatings, and low pH results in both the clay particle and protein having positive charge, so adsorption takes place through cation exchange (Yu et al., 2013). At neutral pH adsorption shifts from Al or Fe-oxides or cation exchange on clays to primarily electrostatic attraction between the protein and clay particle, a weaker bond (Yu et al., 2013). At basic pH, protein molecules and clay particles become more strongly negatively charged, weakening electrostatic attraction and decreasing adsorption (Yu et al., 2013).

Presence of existing N plays a role in adsorption as well, and N-containing DOM adsorption on mineral surfaces has been shown to be related to antecedent SOM (Deb and Shukla, 2011). Kothawala and Moore (2009) observed a negative relationship between soil N and DON adsorption which may be due to increased saturation of protective adsorption sites (Von Lützow et al., 2006). Preferential adsorption of N-containing compounds has been observed through increased concentration of N-rich products at the contact point with mineral surfaces (Bonnard et al., 2012) which agrees with the framework suggesting a layered accumulation of OM on mineral surfaces favoring N-rich compounds on the inside put forward by Kleber (2007, but see Kaiser and Zech, 2000). This layering, with more strongly bonded, N-rich compounds on the inside allows outer compounds to be easily displaced by inputs of fresh OM, leading to decreased N adsorption capacity when attachment sites are saturated (Scott and Rothstein, 2014).

Sorption alone will only protect the portion of the molecule which is directly attached to the mineral particle and which blocks enzyme active sites (Von Lützow et al., 2006). Unattached portions of the protein will remain bioavailable. Dippold et al. (2014) used an incubation experiment to demonstrate that a small amount of strongly sorbed amino acids accumulated on mineral surfaces but that a remarkably large proportion of the sorbed material was still microbially available and was degraded. Vogel et al. (2015)
found that clay minerals with a higher surface area and thus greater number of adsorption sites actually sequestered a smaller amount of N, questioning the importance of the direct adsorption of proteins to mineral surfaces as the dominant sequestration mechanism. Current views are mixed, but although adsorption appears to be important for protecting a smaller amount of N that is attached directly to the mineral surface, on the whole, its primary importance comes from its ability to retard the movement of N within the soil (Vogel et al., 2014, 2015). This allows other retention mechanisms, such as microaggregate formation and spatial separation from microbes to take place.

2.3 Spatial inaccessibility

Occlusion within an aggregate or spatial separation from microbial decomposers effectively preserves N in soil over long periods of time (Von Lützow et al., 2006). Spatial inaccessibility may be the primary driver of N storage by physically protecting OM from microbes and their enzymes through occlusion within an aggregate or within pores which are too small for microbes or their exoenzymes to access, (Marschner et al., 2008). This protection is evidenced by the observation that occluded SOM decomposes at a slower rate and exists in higher concentrations than free SOM (Marschner et al., 2008; Nichols and Halvorson, 2013).

The size of aggregates can be a key predictor of the relative amount of N retained in OM. Microaggregates (< 250 µm) often have a lower C:N ratio than larger size aggregates, reflecting the increased contribution of microbial metabolites protected in this fraction (Nannipieri and Paul, 2009; Hatton et al., 2012; Gleixner, 2013; Pronk et al., 2013). The current prevailing view of aggregate formation begins with the introduction of plant litter to the soil, which is progressively broken down by the soil micro and macrobiota (Hatton et al., 2012). The smaller compounds produced by this microbial processing bind with mineral surfaces, creating high density, highly stable clay and silt-size microaggregates (< 20 µm; Von Lützow et al., 2006; Rillig et al., 2007; Wilson et al., 2009; Hatton et al., 2012). These microaggregates can then combine to form larger microaggregates (20–250 µm), with recent studies highlighting the role of micro-
bacterial necromass in this process (Schurig et al., 2013). Investigations of late hint at the important role cell envelope fragments and polysaccharides can play as a bridge between particles, and these materials may comprise most of the OM attached to mineral surfaces (Dungait et al., 2012; Miltner et al., 2012; Six and Paustian, 2014). Schurig et al. (2013) found that as soil developed following glacial retreat, accumulation of microbial residues on mineral surfaces was likely facilitated by the matrix formed by cell envelope fragments, which eventually coalesced to form microaggregates.

According to current models of aggregate development, the binding mechanisms of microaggregates are generally considered persistent, if not permanent and highly stable, allowing for occlusion to contribute significantly to N storage (Wilson et al., 2009; Nichols and Halvorson, 2013). Almost 50 % of total soil OM has been found to be stored in these small units (Virto et al., 2008). Further aggregation of microaggregates into macroaggregates can occur, especially through the containment of roots and fungal hyphae (Wilson et al., 2009). Stability of these macroaggregates is much more transient however, and unless SOM is continually replenished, aggregate stability will decline (Six et al., 2004; Dungait et al., 2012). Recent research indicates that microbial cell type (i.e. fungi, actinobacteria, Gram-positive or Gram-negative bacteria) does not influence the amount of microbial necromass which is stabilized in soil (Throckmorton et al., 2015).

Physical separation may be especially important in preserving OM in deeper soils where diffusion of nutrients and water to microbial decomposers is more limited (M. W. I. Schmidt et al., 2011; Scott and Rothstein, 2014) although the more stable subsoil environment does provide more favorable climatic conditions for microbes than the relatively more hostile surface soils that experience a greater range of temperature and moisture regimes (Dungait et al., 2012). Microbial substrate limitation due to reduced diffusion can also be enhanced in dense fractions containing high percentages of clay and silt and in subsoils (Marschner et al., 2008). Reduced microbial activity will reduce the amount of N-containing compounds degraded by microbes, but it will also reduce the amount of microbial byproducts produced which form the main constituents
of N that is stored for long periods. Access to the N-containing compounds must be minimized in order for long-term persistence and storage to occur.

3 Policy and management implications

The current understanding of sequestered N as made of chemically labile microbial products, stabilized by adsorption on mineral surfaces and occlusion in aggregate's structure may require an updated management approach to several aspects of N in soils such as saturation processes. It may also have implications for assessing the effectiveness of ecological restoration practices as well as mitigation strategies for reducing anthropogenic N inputs through policy instruments such as critical loads.

Improved appreciation of the factors important for long-term N storage can have implications for ecosystem N saturation and NO$_3^-$ leaching. The influential framework for N saturation put forth by Aber et al. (1998) postulated the orderly transition of an ecosystem subject to enhanced N deposition through stages where NPP, N-mineralization and foliar-N increase with N additions until the system becomes N saturated, at which point NPP and mineralization decrease, while nitrification and NO$_3^-$ leaching take hold. This framework, however, has been refuted by observations that in many cases NO$_3^-$ leaching is one of the first pathways to respond to additional N and that N levels exceeding the critical load do not automatically lead to leaching (Thimonier et al., 2010; Lovett and Goodale, 2011). This led Lovett and Goodale (2011) to propose that N saturation must be considered in terms of capacity (the amount of N which can be retained in soil or biological stocks) as well as kinetics (the rate at which N can be processed).

As we discuss below, the first step in the retention of added N is microbial processing driven by C availability, a driver of kinetic saturation. As the authors note, evidence for capacity saturation on the other hand is weak in most undisturbed temperate ecosystems. Many ecosystems with a variety of vegetation and soils have demonstrated an ability to retain most additional N, with exceptions being areas having obviously low capacity levels such as thin alpine soils (Sogn et al., 1999; Fenn et al., 2003; Johnson...
and Turner, 2014). Given these observations we can conclude that capacity saturation is a state that is rarely reached and there is little evidence of an overall capacity for the retention of N. Upon examination at the fine scale or in the short term though, soils may achieve saturation of adsorption or aggregation sites for N in discrete compartments, depending mainly on mineral composition, location within the soil profile and amount of incoming N and C. Adsorption to mineral surfaces is the first step in long-term N storage and although the amount of adsorption sites will vary with the mineral composition of the soil, it is finite. This is also true for SOC, and forms the basis of the C-saturation model put forth by Six et al. (2002) and observed by Stewart et al. (2007). The saturation of adsorption sites, especially in upper soil horizons where N initially enters the soil, will result in N-compounds bonding weakly to outer OM in the kinetic zone described by Kleber et al. (2007) and observed by Düümg et al. (2012), where hydrophilic (N-rich) molecules are bonded to mineral surfaces but more weakly retained on accreted OM (Bonnard et al., 2012). Fresh inputs of OM will have a higher C : N ratio, closer to that of the original plant material from which it is derived. This more highly hydrophobic material will then displace these weakly-sorbed, outer compounds and allow them to migrate to deeper soil levels where mineral adsorption site capacity is unlikely to be saturated, as illustrated in Fig. 2 (Kaiser and Zech, 2000; Scott and Rothstein, 2014). This mechanism may be responsible for the observation by Castellano et al. (2012) that N-retention decreases as the amount of mineral-associated OM increases, as well as further explain the commonly observed decrease in soil C : N ratio with depth (e.g., Rumpel et al., 2012; Denef et al., 2013). Scaling these capacity considerations from fine scale, homogeneous soil volumes to larger scale or ecosystem wide generalizations is problematic though and the fate of these compounds that move to lower soil horizons is not fully understood. Thus, although capacity saturation may occur in small, discrete areas, its importance to long-term ecosystem N retention is likely to be minimal.

The capacity of a soil to process N is increasingly being recognized as the bottleneck that leads to N saturation; this kinetic saturation appears to be driven by an imbalance
of N inputs over C inputs (which may decrease themselves due to N input effects), but factors that influence long-term N storage may play a role as well (Schimel and Bennett, 2004; Kopáček et al., 2013). Pulses of N released during the initial stages of litter breakdown are retained on adsorption sites which allows for attack by microbial degraders (Hatton et al., 2012). This high N environment, as well as environments with high anthropogenic additions, favor bacterial over fungal communities (Zak et al., 2011). As described earlier, processing by microorganisms represents a crucial step in the creation of sequesterable N so increased microbial processing by bacteria, which have a higher turnover rate and lower C : N ratio than fungi, will increase the production of microbial residues such as cell wall fragments suitable for long-term storage. However, this will be true only up to a point, which may come rapidly as C is consumed. Without a coincident increase in C, microbes will begin to utilize peptides and other small, organic N molecules for C (energy) and excrete excess N as NH$_4^+$ rather than as organic N products or detritus (Farrell et al., 2014). This NH$_4^+$ may subsequently be transformed to NO$_3^-$ and leached from the system in stream water, leading to an interesting conclusion where environments that are able to retain higher amounts of N in the short-term through adsorption may actually reduce the amount of N that is stored in the long-term through an earlier transition to an N saturated state, as discussed above. This pulse dynamic was observed by Lewis et al. (2014) who noted that a greater amount of experimentally added N was rapidly transferred to storage in soils with larger O horizons (greater amount of C for microbial activity). In addition, recent work points to the importance of mycorrhizal fungi in mediating N saturation. When adsorption and supply of N in soil is high, plants decrease their C allocation to mycorrhizal fungi (Bahr et al., 2013). This reduction in C increases microbial reliance on peptides for C and decreases the microbial stoichiometric sink strength, which will decrease long-term N storage (Högberg et al., 2014b).

Although N addition to ecosystems is a natural process, anthropogenic additions, especially from fossil fuel combustion and agricultural emissions from livestock operations, have significantly enhanced rates of N deposition in many areas above naturally
occurring levels (Canfield et al., 2010). In order to address these increased inputs a policy instrument called critical loads was developed. Pioneered in Europe in the 1980’s, they have been used successfully as a means of mitigating the harmful effects of N deposition (Holmberg et al., 2013). A critical load is defined as the amount of deposition below which no significant effects to the ecosystem are thought to occur according to current knowledge, and is meant to inform the amount of N an ecosystem may endure before unwanted effects become manifest (Nilsson and Grennfelt, 1988).

A common method for generating a critical load is the calculation of a simple mass balance (SMB) equation, for example,

\[ \text{CL}_{\text{nut}}(N) = N_i + N_u + N_{\text{de}} + N_{\text{le(acc)}} \]  

where \( \text{CL}_{\text{nut}}(N) \) is the critical load of nutrient nitrogen, \( N_i \) is the long term net immobilization of N in soil, \( N_u \) is the net removal of N in harvested vegetation and animals, \( N_{\text{de}} \) is the flux of N to the atmosphere via denitrification and \( N_{\text{le(acc)}} \) is the acceptable level of leaching loss of N through the root zone (UBA, 2004). This widely used model describes steady state conditions among other simplifying assumptions. Because of these simplifications, using biologically valid values for the terms becomes critical to obtaining a useful result.

The durable immobilization (\( N_i \)) term is one of the least well documented of the SMB equation inputs but is crucial to determining an accurate critical load. In this context, \( N_i \) is the long-term (decades to centuries or longer) accumulation of N in the root zone. In the critical load context, \( N_i \) represents the amount of N which is retained in a naturally functioning ecosystem without inputs of anthropogenic N, and where the soil C : N ratio does not change. This is to be distinguished from long-term N storage, which represents the total amount of N which is able to be stored in all soil profiles, including enhanced accumulation from man-made sources. Despite this difference, the factors governing the transfer of N into the long-term storage pool should be identical. An increased understanding of the factors governing N storage will improve model input estimates for \( N_i \) and thus critical load estimates.
The changed paradigm of N storage in soils also has implications for judging the effectiveness of mitigation strategies. The belief that the long term storage of N in soil was due to chemical recalcitrance and condensation reactions meant that for the most part, the persistent, sequestered pool of N in the soil could be removed only through disturbance or via transport through the soil and subsequent leaching. The new understanding that most N retained in soils is in the form of small, highly labile molecules means that unless well protected from microbial access through protection within the soil matrix, this N can become available again once elevated inputs cease. This can lead to losses of SON, and may lead to losses of N from the ecosystem through nitrification (because NO$_3^-$ is more mobile within soils) and denitrification, especially when C is limiting. Since most N in soils is labile, ecosystems may exhibit signs of elevated N for a period of time after inputs are reduced as the accumulated N is mined. This will be especially true in soils with fewer factors favoring long-term N storage. Ectomycorrhiza have been shown to recover relatively quickly following cessation of N loading and these same fungi are known to mine soil for N when easily accessible inputs do not meet their demand (Högberg et al., 2010; Hobbie et al., 2013). This extraction of stored N may mask the efficacy of mitigation efforts until the system returns to its previously N-limited state. Högberg et al. (2014a) found elevated levels of foliar N even two decades following reduction of N inputs while grasslands studied by Isbell et al. (2013) failed to recover biodiversity losses decades after N-enrichment ended.

Because adsorption is strongly dependent on pH, mitigation measures that increase the pH of the soil, such as controls on sulfate emissions and subsequent deposition, may change the nature or capacity of N adsorption to minerals (Yu et al., 2013). An increase in pH would change the favored adsorption mechanism from ligand exchange with Al and Fe-oxides or cation bridging with clays to electrostatic attraction on layer silicates, a weaker bond, although properties of the protein are important as well (Von Lützow et al., 2006; Rillig et al., 2007). This may increase desorption and accessibility of the molecules to enzymatic attack, however, more research is required to elucidate the magnitude of this effect on long-term N storage.
Finally, identification of the factors important to long-term N storage should enable better prediction of ecosystems that are able to cope with increased N additions, while better informing biogeochemical models. Using $^{15}$N as a tracer, Hatton et al. (2012) found that litter-derived N followed the same pathway through microbes and macroaggregates into microaggregates where it was protected, despite differing soils, bedrock and OM composition. This suggests that the pathways through which N travels on its way to long-term storage, may be similar for different ecosystems, but edaphic factors such as the presence of Fe and Al-oxides, pH and C and N status, and biotic factors such as substrate quality and microbial community composition may regulate the flux of N to (and less importantly the capacity of) long-term storage sites. It was observed by Lewis et al. (2014) that forest soils with greater C content (such as old growth forests) rapidly integrate greater amounts of N into long-term storage than forest soils with lower C contents. This observation strongly indicates that factors which facilitate the sequestration of N may also increase resilience to increased N deposition, an important management consideration in the midst of serious anthropogenic alterations to ecosystems through N deposition.

4 Conclusion

The long-term retention of N in ecosystems is important for many reasons. Although many factors are at play, the overall efficiency with which N is sequestered can contribute to whether an ecosystem is generally N or C-limited, and whether it reaches its capacity to process N, leading to N saturation. The revision of the view of N in soil, from the belief of sequestered N as being chemically recalcitrant and able to resist the attacks of microbes, to a view where in order to persist it must be physically separated from them, has required a reevaluation of the factors that govern N retention. This changed understanding has implications for judging the effectiveness of mitigation measures as well as for our understanding of soil N and C stocks and cycling. For example, if it is true that most N in the long-term storage pool is labile but protected...
from degradation by inaccessibility, than simply measuring the amount of labile N in a soil may overestimate the amount of N in the bioavailable pool and misrepresent the N status of the ecosystem (Darrouzet-Nardi and Weintraub, 2014).

Estimates of the amount of N sequestered in soils have received little attention. Even in Europe where critical loads are used in a regulatory manner, estimates of the amount of N that has accumulated in the long-term lack a consensus; ecosystems in the United States have received even less attention (Duarte et al., 2013). Better understanding of the factors that govern long-term N storage will improve our understanding of N and C cycling and their effects on ecosystem structure and function, and will improve our understanding of saturation processes. It will improve critical load estimates derived from SMB models to help determine acceptable levels of N inputs to ecosystems, and better inform policy makers and land managers when developing strategies for protection, mitigation and restoration of areas experiencing elevated N inputs (Lovett and Goodale, 2011). Building on current knowledge, we have outlined the factors that are important for the incorporation of N to long-term storage (Fig. 1), and outlined a mechanism for N sequestration at depth (Fig. 2), yet much work is needed to detail these processes and examine how the heterogeneous nature of soils, climate and vegetation interact to control this flux. Current levels of anthropogenic N inputs have had lasting effects on ecosystems (Thimonier et al., 2010); a better understanding of long-term N storage and the factors that are important to it will help us determine just how lasting those effects could be and help us be better stewards of our environment.

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References


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Introduction

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Figure 1. Conceptual diagram of the processes involved in N sequestration and the important factors controlling them, and thus having implications for policy and/or management.
Figure 2. Schematic representation of the process by which N would be preferentially sequestered at depth as observed by Scott and Rothstein (2014). Incoming, C-rich, hydrophobic SOM displaces N-rich, hydrophilic SOM that is weakly bound to existing SOM when adsorption sites are saturated. This N-rich, hydrophilic material then migrates lower in the soil profile to where mineral adsorption sites are available.