REVIEW AND SYNTHESIS

Global patterns and substrate-based mechanisms of the terrestrial nitrogen cycle

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Abstract

Nitrogen (N) deposition is impacting the services that ecosystems provide to humanity. However, the mechanisms determining impacts on the N cycle are not fully understood. To explore the mechanistic underpinnings of N impacts on N cycle processes, we reviewed and synthesised recent progress in ecosystem N research through empirical studies, conceptual analysis and model simulations. Experimental and observational studies have revealed that the stimulation of plant N uptake and soil retention generally diminishes as N loading increases, while dissolved and gaseous losses of N occur at low N availability but increase exponentially and become the dominant fate of N at high loading rates. The original N saturation hypothesis emphasises sequential N saturation from plant uptake to soil retention before N losses occur. However, biogeochemical models that simulate simultaneous competition for soil N substrates by multiple processes match the observed patterns of N losses better than models based on sequential competition. To enable better prediction of terrestrial N cycle responses to N loading, we recommend that future research identifies the response functions of different N processes to substrate availability using manipulative experiments, and incorporates the measured N saturation response functions into conceptual, theoretical and quantitative analyses.

Keywords

Leaching, mineralisation, nitrification, nitrogen deposition, nitrogen loss, plant N uptake, saturation, soil N retention, threshold.

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INTRODUCTION

With rapidly increasing rates of fossil fuel combustion and use of synthetic fertilisers, human activities have dramatically accelerated the global fixation and movement of reactive nitrogen (N) (Vitousek *et al.* 1997; Galloway 2005). Increased reactive N loading to terrestrial ecosystems can alter plant growth, soil chemistry and other ecosystem functions (Vitousek *et al.* 1997; Gruber & Galloway 2008). The severity of these impacts is contingent not only on the rate and duration of N deposition, but also on the mechanisms that regulate N dynamics within an ecosystem. Added N through

atmospheric deposition can stimulate plant growth, and be subsequently stored in plants and soil, but it can also be lost through gas emissions and leaching (Aber *et al.* 1989; Lovett & Goodale 2011). The fate of N within an ecosystem has important consequences for the functions and services that ecosystems provide: increased plant N uptake can stimulate plant production in ecosystems, while increased N losses as gas emissions can affect atmospheric chemistry and as leaching can lead to eutrophication of streams and lakes (Aber *et al.* 2003). Prolonged N loading can saturate the capacity of an ecosystem to store N (Ågren & Bosatta 1988; Aber *et al.* 1989). However, the fundamental mechanisms that determine

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the fates of N loading through atmospheric deposition and fertilisation are not well understood. Understanding such mechanisms is critical if we are to develop ecosystem mitigation and adaptation strategies related to N deposition.

Progress has been made in our understanding of ecosystem responses to N loading over the past 30 years. Results from extensive observational and empirical studies form the basis of current conceptual, theoretical and modelling analyses of N impacts on ecosystems. Further, conceptual frameworks have been proposed and many biogeochemical models have been developed over the past decades to characterise ecosystem N dynamics in response to N loading. To date, these three activities – empirical research, conceptual analysis and model development – have all contributed to our understanding but have not been evaluated in concert to identify critical knowledge gaps and future directions for research on terrestrial N dynamics.

This paper comprehensively reviews and evaluates recent progress in ecosystem N research through empirical studies, conceptual analyses and model simulations. We first review observational and experimental results to identify global patterns of plant N uptake, soil N retention and gaseous and dissolved N losses in response to cumulative N loading. These response patterns are then used to evaluate existing conceptual frameworks and biogeochemical models. Review of these conceptual frameworks and models suggests that substrate-based mechanisms have the greatest potential to explain and predict patterns of ecosystem N dynamics. Finally, we highlight research needed to further understand substrate-based mechanisms and develop the modelling capacity to predict ecosystem N responses to N loading.

OBSERVATIONAL AND EXPERIMENTAL EVIDENCE

In recent decades, observational and experimental studies have examined a wide variety of N processes, including plant N uptake, soil N retention and N losses through leaching and gas emissions, in response to N loading. In this section, we build upon previous synthesis studies and assess global response patterns of plant N uptake, soil N retention and N losses. Those global patterns are then used in the following sections to evaluate existing conceptual frameworks and biogeochemical models (Table 1).

Plant N uptake, productivity and foliar N concentrations

Results from observational and experimental studies generally show that plant N uptake initially increases with N addition until N demand by plants is saturated and then levels off (Fig. 1). This pattern was initially observed in an eastern US forest N addition experiment (Aber *et al.* 1998) and further recorded in a global meta-analysis where plant N uptake increased on an average by 48% in response to N addition (Lu *et al.* 2011a) (Fig. 2). The increase in plant N uptake may lead to an increase in plant N concentration, net primary productivity (NPP) or both. Nitrogen concentrations in plant tissues, such as leaves, roots and wood, generally increase with N inputs (Xia & Wan 2008; Liu & Greaver 2010; Crowley *et al.* 2012). For example, a meta-analysis of 304 experimental

studies found that foliar N concentrations increased by an average of 29% (Xia & Wan 2008). Further, the foliar N concentrations in the N-treated stands of an oak forest in southeastern New York initially increased by 20% and then remained stable in a long-term N addition experiment (Lovett & Goodale 2011). An observational study across an N deposition gradient in the northeastern USA also detected an increase in foliar N for the dominant tree species with increasing N deposition (Crowley *et al.* 2012).

Net primary productivity is generally stimulated under elevated N supply. A global meta-analysis of N addition experiments found that aboveground net primary production (ANPP) increased by 29% when N was added (LeBauer & Treseder 2008), and N has been shown to stimulate ANPP across most terrestrial biomes (Lu *et al.* 2011b). In a separate meta-analysis of 304 experimental studies, N additions led to a 54% increase in plant biomass (Xia & Wan 2008). Forest inventory and eddy covariance observations have also shown that forest growth increases under N enrichment in North America (Thomas *et al.* 2010), Europe (Magnani *et al.* 2007; Solberg *et al.* 2009) and East Asia (Yu *et al.* 2014). Together, higher NPP and N concentrations in plant tissues result in more N being incorporated into vegetation pools under elevated than ambient N conditions (Lovett & Goodale 2011).

Tree mortality has been reported to increase under very high N addition levels in long-term N fertilisation experiments. For example, mortality was elevated in a western Massachusetts pine stand (Magill *et al.* 2000), mixed-oak forests in southeastern New York (Lovett & Goodale 2011), a red spruce ecosystem in Mt. Ascutney, Vermont, USA (McNulty *et al.* 2005) as well as in long-term observational studies across European forests (Schulze 1989) under high rates of N deposition. Forest inventory studies have also found that high N deposition reduced the growth of some species (Epa 2008; Thomas *et al.* 2010). Increased mortality and decreased growth rates at high levels of N addition can result from soil acidification and ion imbalance (Wallace *et al.* 2007; Xia & Wan 2008; Tian & Niu 2015), altered competitive interactions and light limitation (Niu *et al.* 2010a). However, because long-term data are scarce, it is difficult to assess whether or not the negative responses of sensitive species to high N deposition are strong enough to affect NPP at regional scales.

Much less studied is how plant N uptake by roots is directly regulated by soil N substrates in the form of NH_4^+, NO_3^- and organic N. Many studies use the amount of N addition or deposition in experiments as an independent variable to characterise the response patterns of plant N uptake under N deposition (Xia *et al.* 2008; Lu *et al.* 2011a). However, plant N uptake by roots does not directly respond to the amount of N addition but to soil N substrates. Functional relationships between plant N uptake and soil N substrates should offer better physiological insights into the kinetics of plant N uptake than the relationship between N uptake and the amount of N added. The kinetics of root uptake with regard to soil N substrates have been characterised for some economically important crops such as wheat (Goyal & Huffaker 1986), barley (Kronzucker *et al.* 1999), rice (Youngdahl *et al.* 1982), tea (Yang *et al.* 2013), eucalypts (Garnett *et al.* 2003) and citrus (Cerezo *et al.* 2007). In general, plant root N

Table 1 The empirical evidence, existing frameworks and model simulations of ecosystem nitrogen dynamics in response to nitrogen loading. The empirical evidence is the observed response across terrestrial ecosystems. The existing frameworks include the original N saturation (Aber *et al.* 1989), the revised N saturation (Aber *et al.* 1998) and the kinetic N saturation (Lovett & Goodale 2011) concepts. Model simulations mainly refer to the current generation that uses the sequential competition scheme to regulate different N processes in response to N loading

		Existing frameworks			
	Empirical evidence	Original N saturation	Revised N saturation	Kinetic N saturation	Model simulations
Foliar N concentration	Increase rapidly first and then slowly	Increase until saturated	Linearly increase with N addition	Increase then remain constant	Increase until saturated to a stable state though many models do not simulate foliar N concentration
NPP or biomass	Increase until saturated with case studies on enhanced plant mortality likely due to other pollutants	Increase until saturated and then decrease	Increase until saturated and then decrease	Increase until saturated	Increase linearly at first and quickly saturate to a stable state. NPP decreases only in some models for tropical forest after N saturation, due to declining carbon use efficiency
Mineralisation	Increase until saturated and then decrease	Linearly increase	Increase until saturated and then decrease	No significant change	Immediately decline. Decline continues in some regions but recovers in others
Nitrification	Exponentially increase with N loading	Exponentially increase after saturation	Exponentially increase after saturation but in small fraction	Exponential increase from the beginning	Some models include nitrification
Nitrate leaching	Occur at early stage of N loading and become the dominant fate at high N loading	Exponentially increase after saturation	Exponential increase after saturation but in small fraction	Increase almost immediately after the start of the N input	Model-dependent
Gaseous loss	Exponentially increase with N loading	Exponential increase after saturation	N/E	Increase even at low levels of N deposition	Model-dependent
Microbial immobilisation	Decrease with N addition	N/E	One possible mechanism for soil N retention	N/E	Implicitly simulated in models
Abiotic N incorporation	Unlikely occur according to ^{15}N data	N/E	One possible mechanism for soil N retention	N/E	Not simulated in models
Mycorrhizal assimilation	Decrease with N addition	N/E	One possible mechanism for soil N retention	N/E	Not explicitly simulated in models
Ca : Al, Mg : N	Base cations decrease with N addition	N/E	Linear decrease with N gradient	N/E	Not simulated in model
Summary of the key points	All N processes respond simultaneously to N addition. Loss is the dominant fate pathways, especially at high N addition rate.	Plant uptake is the main sink. N loss happens only after plant N saturation	Soil N retention is high, most likely due to mycorrhizal assimilation	Added N can flow simultaneously to all sinks and losses in the system, the fate of the added N depends on the strength of the sinks.	Most models simulate plant N demand and immobilisation, which drive soil N dynamics rather than use soil mineral N as substrate to determine relative responses of various soil N processes to N addition.

N/E, not examined.

uptake follows an asymptotic positive relationship with soil mineral N concentration (Hajari *et al.* 2014) (Fig. 3a).

Soil N retention

It is hypothesized that soil N retention occurs through microbial immobilisation of N, physical incorporation of inorganic N and mycorrhizal assimilation of N (Aber *et al.* 1998). A meta-analysis of ^{15}N tracer addition experiments across ecosystems showed that, in the short term (less than a week), 39, 17 and 43% of added ^{15}N tracer was retained in the soil organic layer in forests, grasslands and tundra ecosystems respectively (Templer *et al.* 2012). Over 3–18 months, the largest amount of added ^{15}N to the ecosystem was retained in the organic soil layers in forests (36%) and in the litter layer in grasslands (26%), shrublands (34%) and wetlands (34%). To better understand N partitioning among various processes, we calculated the mass balance of plant and soil pools across all N addition experiments published globally (see detailed methods in supplemental materials). We found that 25% of added N was retained in soil, 9% was retained in aboveground plant tissues, 2% was retained in belowground plant tissues and 2% was retained in plant litter (Fig. 4). Overall, soil
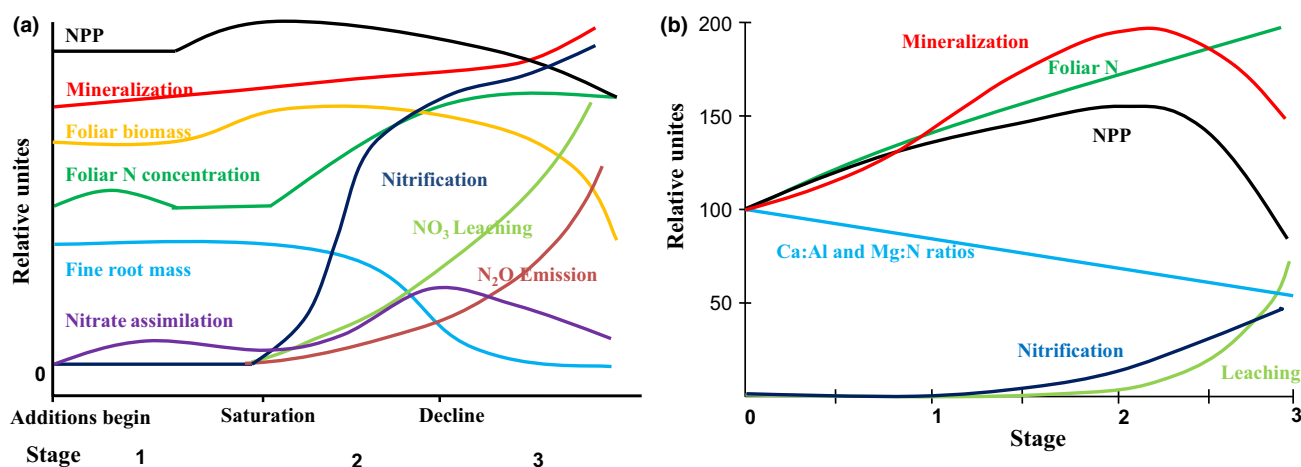
Figure 1 Response patterns of forest N cycling to continuing N addition as hypothesised by Aber *et al.* (1989) (a) and Aber *et al.* (1998) (b). For consistency, the two panels were modified from the corresponding original figures.
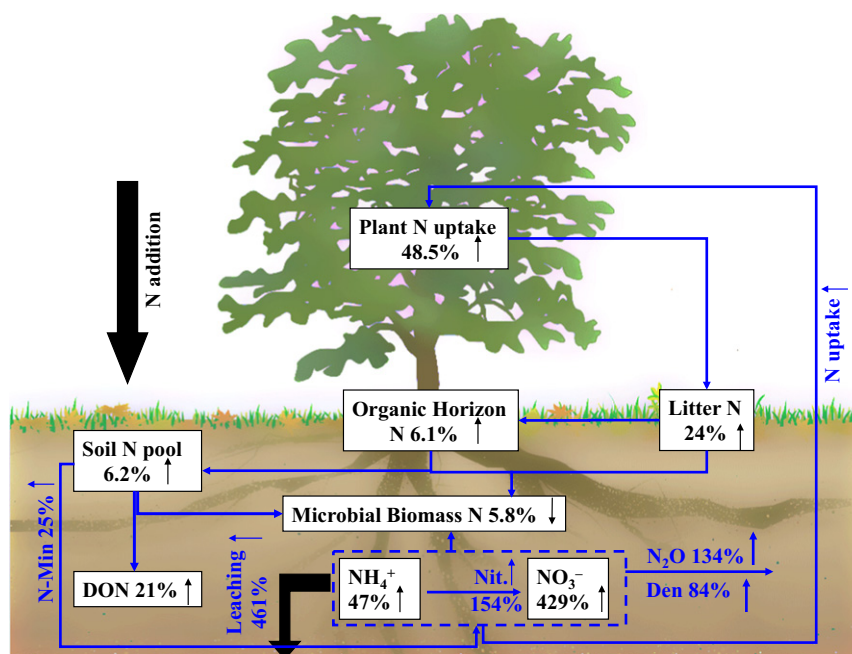

Figure 2 Synthesised responses of ecosystem nitrogen (N) cycling processes to N addition based on a meta-analysis by Lu *et al.* (2011a). ↑ = increase in response to N addition; ↓ = decrease in response to N addition; Nit. = Nitrification; Den. = Denitrification; DON = Dissolved organic N.

retention accounted for less than half of the N loaded into an ecosystem.

Microbial immobilisation is unlikely a mechanism underlying soil N retention as N addition generally results in a decrease in microbial biomass. Microbial biomass decreased by an average of 5.5% under N addition, according to a meta-analysis of 206 published studies (Lu *et al.* 2011a) (Fig. 2). Further, another meta-analysis found that mycorrhizal abundance was 15% lower under N fertilisation (Treseder 2004), indicating that mycorrhizal assimilation is not a common mechanism through which ecosystems store N. Similarly, high levels of N addition can negatively affect soil macrofauna, such as nematodes (Liang *et al.* 2009; Wei *et al.*

2012; Zhao *et al.* 2014), resulting in decreased N storage in soil macrofauna.

A recent global synthesis found that soil inorganic N concentration increased by 114% after N addition, with a 47% increase in the soil NH_4^+ pool and a 429% increase in the soil NO_3^- pool (Lu *et al.* 2011a) (Fig. 2). However, the sizes of these soil inorganic N pools were relatively small in comparison to other pools. As such, increases in soil inorganic N may only account for a small fraction of potential increases in total N retention in soils. In addition, soil inorganic N concentrations can return to ambient levels within two years after cessation of N addition (O'Sullivan *et al.* 2011). This pattern suggests that the inorganic N pools are not stable for N
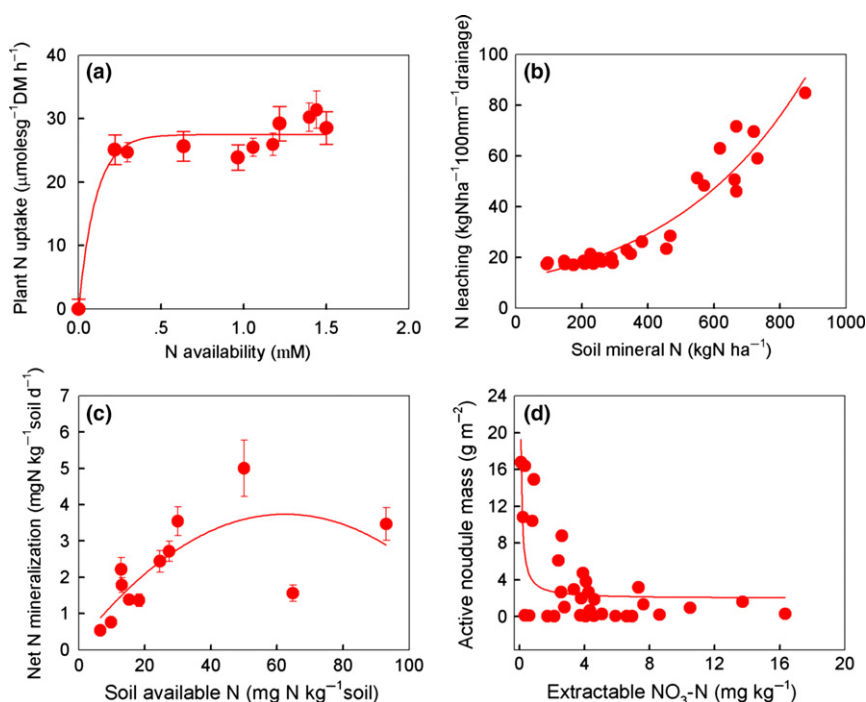
Figure 3 Dependence of various N processes on soil mineral N availability. The data were compiled from Hajari *et al.* (2014) for plant N uptake (a), Di & Cameron (2002) for N leaching (b), Delgado-Baquerizo & Gallardo (2011) for net N mineralisation (c) and Barron *et al.* (2011) for N fixation (d). Detailed methods are in the supplementary materials.

retention in soils. Across studies, increases in N storage in plants and soil are limited in comparison to total N inputs. This imbalance emphasises the importance of N loss processes: leaching and gaseous emission.

N losses

A major unknown, still the focus of many nitrogen deposition studies, is how much N will be lost from terrestrial ecosystems as deposition rates change. Experimental evidence indicates that N losses increase with N addition, even at very low levels of N loading. For example, increases in N leaching and N_2O emission were observed in the very early stages of an N addition experiment, even at N-limited sites (Magill *et al.* 1997, 2004). Moreover, N_2O emissions increased exponentially with the amount of N loading for most crop types after synthetic N fertilisers were applied (Shcherbak *et al.* 2014). In addition, experimental studies found that N leaching and volatilisation exponentially increased with increasing levels of soil mineral N substrates (Di & Cameron 2002) (Fig. 3b).

Observations along N deposition gradients similarly showed that N losses occur even at low levels of N loading. In a survey across the northeastern USA, nitrate leaching increased when atmospheric N deposition rate was above 8 kg ha^{-1} year^{-1}(Aber *et al.* 2003), a level that has already been exceeded in many highly N-polluted areas (Penuelas *et al.* 2013). Similarly, across 121 forested sites in Europe, N loss was greater than retention at sites with N deposition rates above 8 kg ha^{-1} year^{-1} (Dise *et al.* 2009). Further, nitrate leaching into streams has significantly increased over the last two decades in many rural forests of the northeastern USA

(Peterjohn *et al.* 1996; Lovett *et al.* 2000; Burns & Kendall 2002), where rates of atmospheric N deposition were high (> 10 kg N ha^{-1} year^{-1}) (Weathers *et al.* 2006). In unpolluted ecosystems, leaching of inorganic N may be minor, but leaching of organic N can be substantial (Hedin *et al.* 1995). Stable isotope analysis of nitrate in streams and soil solution showed that N exported from forested watersheds throughout the northeastern USA was derived primarily from microbial production (nitrification) (Burns & Kendall 2002; Pardo *et al.* 2004; Templer *et al.* 2015). These results suggest that much of the enhanced N leaching under N deposition results from the cycling of N through organic and inorganic pools, rather than from its passing directly from the atmosphere to streams.

High N losses from ecosystems have been shown to be accompanied by enhanced nitrification and denitrification rates (Liu & Greaver 2009; Lu *et al.* 2011a; Shcherbak *et al.* 2014; Fang *et al.* 2015). For example, a global synthesis of 206 studies found that N addition increased nitrification rates by an average of 154% and denitrification rates by 84% across ecosystems (Fig. 2). From an Earth-system perspective, when all estimated losses of N from terrestrial systems are subtracted from estimated N inputs, the remaining N retained in ecosystems is small (Gruber & Galloway 2008; Schlesinger 2009). Thus, terrestrial ecosystems generally appear to be 'leaky' enough to balance N addition from fertilisation and deposition with leaching and gaseous emissions.

In sum, numerous empirical studies reveal global response patterns of ecosystem N processes to N loading. Plant N uptake and growth are generally stimulated by low levels of N addition, level off at intermediate N levels of N addition and occasionally decline at high N levels of N addition when soil
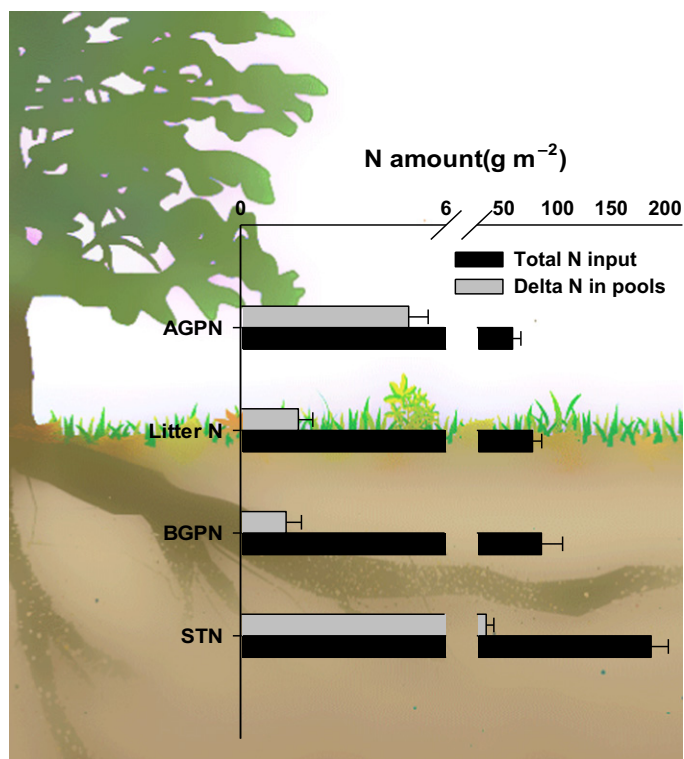
Figure 4 Increments of the amount of nitrogen (N) in different ecosystem pools vs. the total amount of N added across N addition experiments conducted in the world. The data were compiled from 206 published papers obtained from Lu *et al.* (2011a) (See methods in supplementary materials). STN = soil total N; AGPN = aboveground plant N; BGPN = belowground plant N; LN = litter N. Delta N means the increments of N pool size under N addition.

acidification and/or plant mortality occurs. At very low levels of N loading, soil N retention accounts for a substantial portion of the added N, while N losses through leaching and gaseous emissions are accompanied by plant N uptake and soil retention. However, N loss pathways become dominant at high N addition levels (Table 1). Less studied so far are substrate-based mechanisms for N retention and loss, which are crucial for the development of general theory, as well as for models that predict ecosystem responses to N addition.

The roles of ecosystem type, management and climate change

Responses of individual terrestrial ecosystems to N addition may vary because background N levels, N retention capacity, soil organic carbon (C) content and plant community composition can all impact an ecosystem's response to N loading (Evans *et al.* 2006; Vitousek *et al.* 2010). For example, N saturation thresholds for plants in N-limited, semi-arid grasslands may be higher than the thresholds for mesic ecosystems because water deficiency in semi-arid ecosystems may suppress plant N uptake (Bai *et al.* 2010). Moreover, our mass balance calculations across all the N addition experiments suggest that N additions lead to greater retention of litter and plant N in agricultural ecosystems than in non-agricultural ecosystems, and smaller gains in soil inorganic N in agricultural than non-agricultural ecosystems (Table S1) (Lu *et al.* 2011a). The analy-

sis also suggests that forested ecosystems retain less N in aboveground plant, litter and soil pools than non-forest ecosystems (Table S1). Management practices of specific ecosystems can also regulate ecosystem responses to N addition (de Vries *et al.* 2012a). Undisturbed grassland systems are much more sensitive to N addition than grazed ecosystems because the undisturbed sites generally have greater vegetation cover and plant productivity, and thus may have greater potential for plant N uptake (Bai *et al.* 2010). The effects of management (or land use change) on N retention can be related to shifts in soil food web composition. Extensively managed grasslands with fungal-based soil food webs and tight linkages between plant and soil microorganisms are more efficient in retaining N than intensively managed agricultural systems with bacterial-based food webs (de Vries *et al.* 2012a,b).

Climatic change may directly affect ecosystem N responses. For example, warm and moist conditions favour plant N uptake (Melillo *et al.* 2011), N loss from fertiliser application through volatilisation (Bouwman *et al.* 2002), and N use efficiency (Niu *et al.* 2010b). Climate change may also alter an ecosystem's response to N addition through its indirect influence on plant and soil organism composition and interactions (Kardol *et al.* 2012). For example, elevated atmospheric CO_2 concentrations may promote plant productivity and lead to progressive N limitation (Luo *et al.* 2004; McMurtrie *et al.* 2008; Norby *et al.* 2010). In turn, this may increase the N saturation threshold because more N would be incorporated into plant biomass (Bradford *et al.* 2012), leading to reduced N leaching rates (Luo *et al.* 2006). On the other hand, warming may stimulate soil microbial and faunal activity and increase decomposition and N mineralisation rates (Fierer *et al.* 2005; Dieleman *et al.* 2012), which may initially result in increased plant N uptake, but ultimately promote N loss through leaching. Changes in precipitation might shift plant N uptake, especially in dry areas where plants are water-limited (Knapp *et al.* 2008). Thus, the manner in which different N pathways respond to N enrichment will depend on the direct and indirect influences of other global change factors. An important future challenge lies in disentangling the interactive effects of multiple simultaneously changing climate variables.

EXISTING CONCEPTUAL FRAMEWORKS

Over the last three decades, various conceptual frameworks have been developed to characterise ecosystem N responses to N loading. Those frameworks include N saturation, kinetic N saturation and substrate-consumer reaction networks. The 'N saturation' framework describes how ecosystem N dynamics respond to accumulated N deposition (Aber *et al.* 1989, 1998). The original N saturation framework distinguishes three stages of N response in sequence: the primary stage, when plants take up N at low levels of deposition; the mid-stage, when cumulative N storage leads to the saturation of abiotic and biotic N sinks; and the late stage, when enhanced N mineralisation stimulates NO_3^- leaching, N gaseous loss, acidification and eventually reduces plant production due to nutrient imbalances (Aber *et al.* 1989) (Fig. 1a, Table 1). While this original N saturation framework guided much of the early work on N loading, it was revised to emphasise

observations of soil N retention emerging from research in New England temperate forests (Aber *et al.* 1998). The revised N saturation framework, together with newly collected data, affirmed that N retention efficiency of forests was higher than originally predicted because plant–microbe symbioses helped retain nutrients in soils beyond that originally considered (Fig. 1b, Table 1).

The N saturation framework was developed based on the observations from forest N addition experiments in the eastern USA (McNulty *et al.* 1991, 1996; Aber *et al.* 1998; Magill *et al.* 2004; Lovett & Goodale 2011). This framework has since been tested with experimental and observational results from different regions of the world. In general, the responses of plant N uptake and foliar N concentration to N addition have been consistent with both the original (Aber *et al.* 1989) and revised (Aber *et al.* 1998) N saturation frameworks (Table 1). As ecosystems have approached N saturation, NPP responses have levelled off (Magill *et al.* 2000; Bai *et al.* 2010; de Vries *et al.* 2014). The observed levelling off of NPP has likely been a consequence of increased plant mortality and/or decreased plant growth rates and productivity at the very high N levels (Magill *et al.* 2000), but different mechanisms may cause these changes in plant productivity/mortality to emerge (e.g. limitation by other nutrients, especially phosphorus (Penuelas *et al.* 2013; Li *et al.* 2016)). In contrast, mechanisms underlying observed soil N retention may not be the same as those proposed in the N saturation framework. Across many studies, results have not supported the idea that microbial immobilisation and mycorrhizal assimilation are major pathways for retention of added N in soil pools (Treseder 2004; Lu *et al.* 2011a). Moreover, the observed patterns of gaseous and dissolved N losses have differed considerably from the three-stage sequential hypothesis of the N saturation framework (Aber *et al.* 1989, 1998).

More recently, the kinetic N saturation framework was proposed based on the mass balance between N inputs and N outputs (Lovett & Goodale 2011). The kinetic N saturation framework distinguishes 'kinetic' N saturation from a 'capacity' N saturation. It emphasises that N losses occur when uptake kinetics are saturated (i.e. N sink strength) rather than when N storage capacities are saturated (Lovett & Goodale 2011). When N storage capacities are saturated, plants and microbes have no net gain in N. Thus, the kinetic saturation framework suggests that each component of the N cycle responds simultaneously to N addition and thus omits the three-stage sequential response pattern proposed in the N saturation framework (Table 1). Compared to the N saturation framework, the kinetic N saturation framework may do a better job describing observed patterns of ecosystem responses to N deposition, especially the patterns found in eastern US forest ecosystems (McNulty *et al.* 1991, 1996; Aber *et al.* 1998; Magill *et al.* 2004; Lovett & Goodale 2011). However, unlike the N saturation framework it cannot predict temporal patterns of various processes unless the relative strengths of those processes are known (Lovett & Goodale 2011). To fully test the kinetic framework, the relationships among N cycling processes, such as plant N uptake, soil N retention and N losses, with N loading must be quantitatively evaluated.

Another recently developed framework is the 'multiple-substrate–multiple-consumer reaction network' (Tang & Riley 2013). Biotic and abiotic agents such as plant roots, decomposing microbes, nitrifiers, denitrifiers and mineral surfaces that use soil nutrients as substrates for their biological or chemical reactions are the N 'consumers' in this framework. The substrate-consumer framework and the kinetic N saturation framework are conceptually similar because they both have multiple N pathways that are simultaneously affected by soil N substrates. Each of these consumers in the substrate-consumer framework competes for multiple soil N substrates and the success of any consumer in substrate acquisition is affected by its consumer-substrate affinity (Nedwell 1999; Bonachela *et al.* 2011). In response to soil inorganic N and phosphorus addition, the substrate-consumer framework can simulate N dynamics (Zhu *et al.* 2015) as well as account for soil N losses (Zhu & Riley 2015). One of the important assumptions of this framework is that the substrate-consumer relationships follow Michealis–Menten kinetics. However, empirical results indicate that N leaching and gaseous emissions increase exponentially with N substrate (Fig. 3b).

In sum, each of the described frameworks has strengths and weaknesses. The N saturation framework emphasises the temporal dynamics of N saturation sequentially from plants to soils before the occurrence of N leaching and gaseous losses. While there is a lot of empirical plant uptake evidence for the N saturation framework, the sequential order of the occurrence of N losses is not supported by experimental and observational data. The kinetic saturation framework highlights that added N can simultaneously flow to four pathways: plant uptake, soil retention, leaching of dissolved N and emission of gaseous N. Thus, the kinetic saturation framework can explain the observed simultaneous changes in different N pathways better than N saturation framework. While the substrate-consumer framework is conceptually similar to the kinetic saturation framework, it may do a better job describing N dynamics because it more explicitly emphasises soil N substrates for which multiple consumers simultaneously compete. Overall, the substrate-consumer and kinetic saturation frameworks generally better explain the observed response patterns of ecosystem N processes to N addition than the N saturation framework as discussed above.

SIMULATION MODELS

Modelling studies bolster empirical research results by providing longer term insights into ecosystem N processes in response to N loading (Thomas *et al.* 2013, 2015). Most of the coupled C and N biogeochemical models include plant N uptake, soil organic N decomposition, microbial N mineralisation and immobilisation, biological N fixation and different pathways of N export (Prentice 2008). All models follow the mass balance principle where the inorganic N added to an ecosystem equals the cumulative changes in plant N uptake, soil N retention and N loss (through leaching and gaseous emission) (Zaehle & Dalmonech 2011). Nitrogen entering ecosystems via deposition and fertilisation moves into inorganic N pools in the soil before various fate processes consume it.

Most biogeochemical models use a sequential competition scheme to simulate responses of ecosystem N cycling processes to N loading. The models first simulate N demands by plant uptake and soil retention through immobilisation. The simulated N demands then sequentially dictate soil ammonium and nitrate dynamics. Thus, those soil N substrates do not simultaneously regulate various soil N processes (Table 1). For example, the global O-CN model (Zaehle & Friend 2010; Zaehle *et al.* 2010) gives microbes and plants preferential access to mineral N, while N leaching depends on residual mineral N concentrations and N gaseous loss was simulated as a function of nitrification and nitrate concentration. In contrast, another global model, CLM-CN4.0, assumes that microbial N immobilisation and plant N uptake first access soil mineral N in proportion to their demand. A component of N gaseous loss is then modelled as a constant fraction of net mineralisation rate, and leaching finally has the lowest priority to access the residual nitrate (Thornton *et al.* 2009). As a result, model simulations tracing the fate of added N resulted in a larger fraction being retained in plant biomass than in soil in the CLM-CN4.0 model, whereas in the O-CN model, most of the added N was retained in the soil (Thomas *et al.* 2013). The O-CN model simulated leaching of much of the added N, whereas the simulated leaching by CLM-CN was functionally zero (Fig. 5).

In contrast, the substrate-consumer framework employs a simultaneous competition scheme. This competition scheme has been developed into a Nutrient COMpetition model (N-COM) with three soil nutrients (NH_4^+, NO_3^- and POx) and five potential competitors (plant roots, decomposing microbes, nitrifiers, denitrifiers and mineral surfaces) (Zhu *et al.* 2015). N-COM simulates soil N dynamics based on the equilibrium chemistry approximation (ECA) (Tang & Riley 2013) to quantify kinetics of three soil nutrients (NH_4^+, NO_3^{-1} and POx) as substrates to simultaneously regulate plant root uptake, microbial decomposition, nitrification, denitrification and mineral absorption (Zhu *et al.* 2015). The N-COM model can do a better job of simulating observed N losses than CLM4.5, a model that contains the sequential competition scheme (Zhu & Riley 2015). However, N-COM assumes that all substrate-consumer relationships follow Michaelis–Menten kinetics, a pattern not observed in experimental results, where dissolved N leaching follows an exponential function with soil N substrate (e.g. Fig. 3b). Different response functions describing N-consuming processes across different substrate concentrations likely result in different behaviours of modelled ecosystem N dynamics. Thus, incorporation of observed substrate-consumer relationships has the potential to improve model performance.

In sum, the representations of ecosystem N cycling processes vary considerably among biogeochemical models. Most N simulation models use a sequential competition scheme that relies on demands of plant and microbial N uptake to regulate key soil N processes. As a consequence, hydrological N losses or gaseous N emission are usually not well simulated in comparison with observations. In contrast, the substrate-consumer models use a simultaneous competition scheme in which multiple soil N substrates simultaneously regulate multiple N processes, and thus these models simulate the loss processes better than the sequential competition models. Even so, the substrate-consumer model can be further improved if observed functional relationships between substrates ad consumers can be incorporated to represent realistic dynamic patterns.

SUBSTRATE-BASED MECHANISMS

Our review highlights that experimental and observational results do not offer much empirical evidence to support the sequential occurrence of N saturation from plant uptake to soil retention before N loss as hypothesised by the N saturation framework. Both the kinetic saturation and substrate-consumer frameworks have the potential to better describe the observed response patterns that added N as substrate simultaneously regulates plant uptake, soil retention, and dissolved and gaseous losses. Coincidently, the majority of biogeochemical models that use a sequential competitive scheme to consume soil mineral N does not simulate N leaching and gaseous emission well. In contrast, simultaneous competition for N substrates by multiple N fate processes, as in the substrate-consumer model, can simulate N dynamics much better. Thus, the conceptual analysis and modelling studies all suggest that substrate-based mechanisms have the potential to explain and realistically model observed patterns of terrestrial N cycling.

Generally, atmospheric N additions via deposition or N fertilisation increase soil inorganic N in the form of NH_4^+ and NO_3^-. Soil NH_4^+ and NO_3^- are substrates for many N processes, such as plant uptake, leaching, immobilisation and volatilisation. Thus, as described in the kinetic saturation framework, the increased inorganic N supply under N fertilisation or deposition simultaneously stimulates plant N uptake, soil N retention and losses to different degrees (Fig. 3). When multiple N-consuming processes compete simultaneously for common substrates, multiple-substrate–multiple-consumer reaction networks are formed, as described by the substrate-consumer model.

To further explore the role that substrate-based mechanisms play in ecosystem N dynamics, we need to characterise the response functions for leaching, plant uptake, immobilisation, volatilisation, mineralisation and biological N fixation to soil N substrate availability. The substrate response functions are fundamental to understand how mineral N as substrates regulates different N processes. Different N substrate-response functions have been documented for different N processes (Fig. 3) in previous studies. Plant N uptake, for example, follows asymptotic response function to N substrates (Hajari *et al.* 2014) but N loss exponentially increases with N substrates (Di & Cameron 2002). N fixation declines with N substrates (Barron *et al.* 2011), while N mineralisation follows a hyperbolic function (Delgado-Baquerizo & Gallardo 2011). When those response functions are used in a model, such as the substrate-consumer model, different ecosystem N dynamics are likely to be well predicted.

Characterising the N response functions with soil NH_4^+ and NO_3^- concentrations in field studies is extremely challenging as NH_4^+ and NO_3^- turn over extremely fast. Innovative experiments that directly manipulate soil N sub-
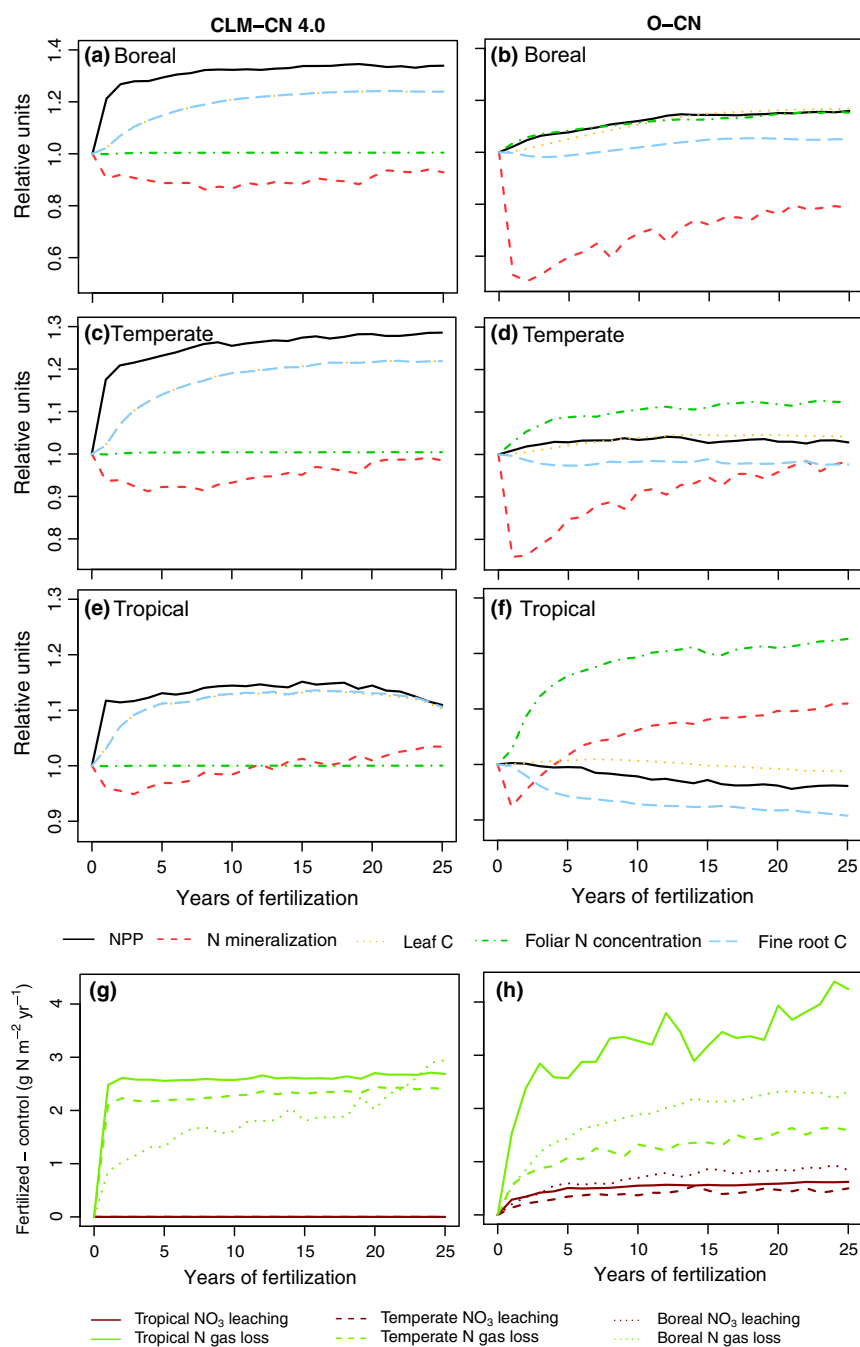
Figure 5 Modelled responses of various nitrogen (N) processes to N fertilisation in different types of forests at a rate of 6 g N m^{-2} year^{-1} for 25 years by two land surface models: CLM-CN 4.0 (a,c,e,g) and the O-CN (b,d,f,h). Panels g and h for N loss processes. The units are expressed as 'Relative Units' to follow the figures in Aber *et al.* (1998). All units are the proportion change over a control simulation except for leaching and N gas emissions (right axis), which are the absolute difference (g N m^{-2} year^{-1}). A description of the models and simulations can be found in Thomas *et al.* (2013).

strate levels to quantify the response functions are needed. We also need to develop mass balance approaches to quantify allocation of the added N to different pools and pathways over longer times scales, as experiments that manipulate N substrate levels generally occur over relatively short periods of time. [15]N labelling has been an effective tool for partitioning added N to different pathways. However, labelling experiments are usually run for less than 18 months, which is too short a time period to make long-term predictions

(Templer *et al.* 2012). In sum, we emphasise the need to develop innovative techniques to examine long-term N partitioning to different pathways and quantifying the ratio of N retention/addition.

Different N partitioning functions determine patterns of ecosystem N dynamics and thus influence N management. For example, when N fertilisation or deposition increases soil N substrate availability, leaching and volatilisation often proportionally or exponentially increase, whereas plant uptake

and soil retention tend to saturate (Fig. 6). As a consequence, more N is likely lost from the ecosystem than retained in plants when N is added to the soil. Modelling analysis to determine the critical point of N substrate level where losses exceed plant uptake, as is shown conceptually in Fig. 6, has important implications for agricultural management and climate change mitigation. If the soil mineral N exceeds the critical point under N fertilisation, N loss may dramatically increase, whereas plant N uptake may be less affected. Fertiliser use strategies based on this framework may help reduce N loss while meeting the N demands for crop growth. Thus, quantifying this critical point and exploring its regulating factors are critical goals for future research.

Substrate-based mechanisms may help to improve a models capability to predict N dynamics under global change. The N-COMM modelling studies show that substrate-based, simultaneous competition schemes can do a better job simulating N dynamics observed in studies, especially dissolved N losses, than the sequential competition scheme (Zhu *et al.* 2015). Model performance may be improved further if the response of various N processes to substrates can be derived from experimental studies and then incorporated into models.

CONCLUSIONS

In this paper, we reviewed and evaluated recent progress in understanding N dynamics in terrestrial ecosystems that has been made through empirical studies, conceptual analyses and biogeochemical modelling. While much progress has been made, experimental evidence, conceptual analyses and modelling activities are not always well integrated and/or coordinated. Globally, the stimulation of plant N uptake and soil N retention appears to diminish with increasing N fertilisation, whereas N losses through leaching and gaseous emissions appear to increase with N addition. While these observed patterns can be partially explained by the N saturation framework, other more comprehensive frameworks do a better job describing N dynamics. For example, the kinetic N saturation and substrate-consumer frameworks, which are based on simultaneous stimulation of different N processes

by increased availability of NH_4^+ and NO_3^- in soil, do a better job describing the observed responses. In addition, the current generation of biogeochemical models mostly relies on a sequential competition scheme where the demands of plant N uptake and soil retention, instead of soil N substrates, regulate soil N processes. The low performances of biogeochemical simulation models in comparison with observations make it necessary to search for alternative modelling approaches (Thomas *et al.* 2013). For example, the N-COM model based on the simultaneous competition scheme can better simulate N losses than CLM4.5 with a sequential competition scheme.

Our review suggests that the substrate-based mechanisms are a better scheme for models to simulate N dynamics in terrestrial ecosystems. To fully understand the substrate-based mechanisms, we need to design experiments that characterise the response functions of different N processes with soil N substrates and quantify long-term partitioning of the added N to different N pathways. The integration of substrate-based mechanisms into biogeochemical models, especially with observed response functions of N processes with substrates from experimental studies, has the potential to improve model capability to predict future N dynamics in terrestrial ecosystems in response to increasing N deposition.
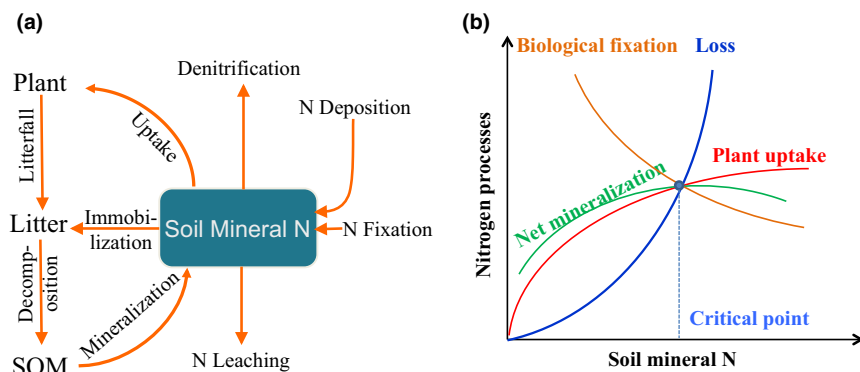
Figure 6 Conceptual diagram illustrating how different components of the nitrogen (N) cycle are related to soil mineral N concentration. (a) Soil mineral N is taken up by plants, lost through leaching and gaseous emission and immobilised by the microbial community and soil physical processes. Soil mineral N also influences biochemical N fixation. (b) The functional relationships between soil mineral N and N cycling processes likely determine different responses of individual processes to N addition in an ecosystem.

AUTHOR CONTRIBUTIONS

This review was conceived at a workshop and all authors participated in the subsequent discussions and planning. S.N., Y.L., J.S., D.T., Q. T. and J.X. were responsible for analyses and figures, and S.N. wrote the first draft of the paper. All authors provided input to the drafting and final version of the manuscript.

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