



## Review

## Going where no grains have gone before: From early to mid-succession



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## ABSTRACT

Annual-based arable agroecosystems experience among the greatest frequency, extent and magnitude of disturbance regimes of all terrestrial ecosystems. In order to control non-crop vegetation, farmers implement tillage practices and/or utilize herbicides. These practices effectively shift the farmed ecosystems to early stages of secondary succession where they remain as long as annual crops are grown. Humanity's long-standing dependence on a disturbance-based food and fiber producing ecosystem has resulted in degraded soil structure, unsustainable levels of soil erosion, losses of soil organic matter, low nutrient and water retention, severe weed challenges, and a less-diverse or functional soil microbiome. While no-till cropping systems have reduced some hazards like soil erosion, they remain compromised with respect to ecosystem functions like water and nutrient uptake, and carbon sequestration compared to many later successional ecosystems. Recent advances in the development of perennial grain crop species invite consideration of the ecological implications of farming grains further down the successional gradient than ever before possible. In this review, we specifically explore how the nitrogen (N) economy of a mid-successional agroecosystem might differ from early-successional annual grain ecosystems as well as native mid-successional grassland ecosystems. We present a conceptual model that compares changes in soil organic matter, net ecosystem productivity, N availability, and N retention through ecosystem succession. Research from the agronomic and ecological literatures suggest that mid-successional grain agriculture should feature several ecological functions that could greatly improve synchrony between soil N supply and crop demands; these include larger active soil organic matter pools, a more trophically complex and stable soil microbiome that facilitates higher turnover rates of available N, greater N retention due to greater assimilation and seasonal translocation by deeply rooted perennial species as well as greater microbial immobilization. Compared to native mid-successional grasslands that cycle the majority of N required to maintain productivity within the ecosystem, a mid-successional agriculture would require greater external N inputs to balance N exports in food. Synthetic N fertilizer could make up this deficit, but in the interest of maximizing ecological intensification in order to minimize inputs and associated environmental consequences, we explore making up the N deficit with biological N<sub>2</sub> fixation. The dominant approach to addressing problems in agriculture is to target specific shortcomings such as nutrient retention or weed invasion. Moving agriculture down the successional gradient promises to change the nature of the ecosystem itself, shifting attention from symptom to cause, such that ecological intensification and provision of a broader suite of ecosystem services happen not in spite of, but as a consequence of agriculture.

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## 1. Introduction

In contrast to native ecosystems, agricultural ecosystems tend to include far fewer species of plants and animals. Agroecologists have recognized this distinction for some time, and the topic of how much and what type of planned agrobiodiversity would improve the functionality and ecological intensification of agriculture continues to receive a great deal of attention (Bommarco et al., 2013; Lin, 2011; Swift et al., 2004). A second broad distinction between native and agroecosystems—one that has received far less attention from agroecologists—is that of succession. Following disturbance, native ecosystems regain functionality through successional changes that strengthen a range of internal, regulating feedbacks. In contrast, due to recurring tillage events or herbicide applications, annual crop ecosystems remain arrested in a disturbed, less regulated state of early secondary succession (Smith, 2014). As a result, degrading processes of soil erosion (Montgomery, 2007), nutrient and water leaching (MEA, 2005; Vitousek and Reiners, 1975), soil organic matter decline (Davidson and Ackerman, 1993), and extensive weed establishment (Liebman and Mohler, 2001) compromise the agroecosystems themselves as well as ecosystems situated down wind, hill or stream. Under these conditions, the opportunities for achieving production goals through ecological intensification are limited (Tittonell and Giller, 2013).

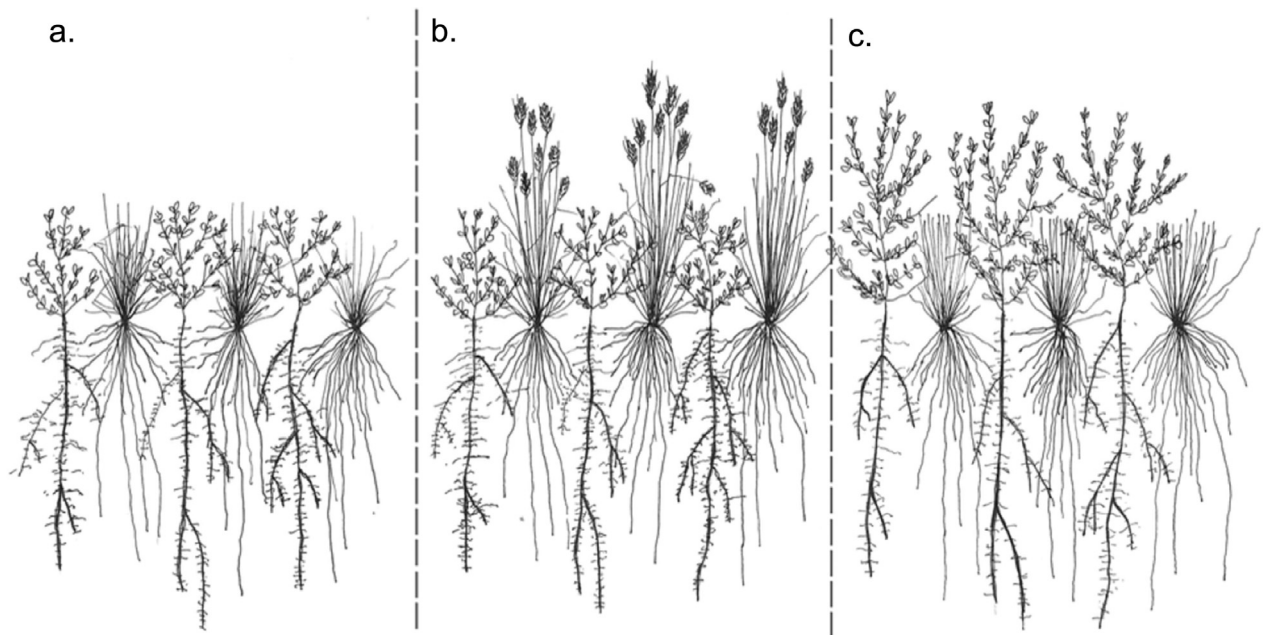
In attempts to rein in the consequences of chronic perturbation, agronomists and ecologists have developed cropping systems that attempt to maximize continuous plant cover on the landscape through cover crops or integration of perennial buffer strips or forage crops (Blesh and Drinkwater, 2013; Liebman et al., 2013). These systems have demonstrated improvements in nutrient retention, carbon (C) accumulation and weed suppression through reduction of soil disturbance and vegetation replacement, and there is good reason to incentivize their adoption. However, these efforts fall short of addressing the root of agriculture's successional stagnation. Critical to the development of numerous ecosystem functions in native ecosystem succession—indeed critical to succession itself—is the transition from community dominance by annual to perennial plant species (Connell and Slatyer, 1977). The prospect of establishing a parallel successional trajectory in agriculture could be transformative (Fig. 1). To this end, breeding

programs in multiple countries are now developing hybrid plant populations or new domestications of perennial grain crops, with promising early results for perennial wheat (*Triticum* spp. × *Thinopyrum* spp.), rice (*Oryza sativa* × *O. longistaminata*), sorghum (*Sorghum bicolor* × *S. halepense*), pigeon pea (*Cajanus cajan*) and oilseeds (Batello et al., 2013; Kantar et al., 2016).

A perennial crop agriculture that exists in a later stage of succession is predicted to change – in some cases dramatically – with respect to multiple agroecosystem processes and attributes including soil and nutrient retention, C sequestration, water infiltration and uptake efficiencies, weed suppression, phosphorus (P) and N availability and soil structure (Glover et al., 2007; Robertson et al., 2011). All of these merit consideration, but here we focus on how the N economy of a mid-successional agroecosystem might change across successional seres, highlighting differences between perennial and annual agroecosystems, as well as unique positive and negative attributes of a mid-successional ecosystem that have yet to be considered in an agricultural context. Although we focus primarily on N, we also examine ecosystem attributes and feedbacks that govern the N economy such as changes in soil C balance, microbiome, and dominant forms of soil P.

### 1.1. Why nitrogen?

The importance of N in sustaining food production, and the serious challenges faced by farmers to manage N resources efficiently make it a salient topic in the context of disturbance and succession. Nitrogen is the nutrient that most commonly limits the productivity of agroecosystems and, either alone or with P, native terrestrial ecosystems (Robertson and Vitousek, 2009; Vitousek and Howarth, 1991). Yet on average, only 30–50% of N applied is recovered in a fertilized grain crop, and beyond that, <7% of the applied N is recovered in up to six subsequent crops (Gardner and Drinkwater, 2009; Ladha et al., 2005). Low N fertilizer uptake efficiencies, caused by the application of high concentrations of the most soluble N forms to fields at times when annual crop roots are either underdeveloped or not present at all, result in substantial N losses to the environment (Robertson et al., 2011). Nitrate-N and to a lesser extent dissolved organic N is lost to surface or groundwater via hydrologic pathways, causing local contamination of



**Fig. 1.** Example of a perennial grain-legume intercrop through a growing season. (a) **Spring.** The two species break winter dormancy producing similar aboveground biomass. (b) **Early summer.** The canopy of the grain crop overshadows and suppresses the legume. **Late summer.** Following grain harvest, light reaches the legume inducing a surge of growth and  $N_2$  fixation. Livestock grazing or mowing may be employed to manage biomass accumulation.

freshwater sources (Townsend et al., 2003), or eutrophication of downstream marine ecosystems (Rabalais et al., 2007). In addition, N is lost from fertilized croplands in the form of greenhouse gases such as ammonia and nitrous oxide (Robertson et al., 2012).

## 1.2. Why legumes?

Replacing single species stands of annual grains with perennial grains would substantially reduce N losses, at least through nitrate leaching, and associated environmental impacts (e.g., Culman et al., 2013). However, single species perennial stands would also require exogenous N to sustain, and reliance on synthetic fertilizer as the primary N input carries many direct and indirect costs. Synthetic N fertilizers constitute the greatest input of commercial energy (typically fossil fuels ranging from natural gas to coal) into industrial agriculture (Smil, 2001). Globally, about 1.3% of all commercial energy resources are expended in N fertilizer production (Crews and Peoples, 2004), and the associated  $CO_2$  emissions can significantly offset any potential C sequestration in soil from alternative land-use practices such as shifting to perennial crop species (Schlesinger, 1999). Symbiotic  $N_2$  fixation by legumes and associated rhizobia bacteria can provide substantial N inputs to agroecosystems using solar rather than fossil energy (Crews and Peoples, 2004; Herridge et al., 2008). This can be a significant factor in the ability for many farmers in less developed countries to obtain costly N inputs (Vitousek et al., 2009).

The updated Planetary Boundary analysis (Steffen et al., 2015) estimates that the input of new reactive N (synthetic and agricultural  $N_2$  fixation) should be limited to about 40% of present use in order to be within the safe operating space in relation to intrinsic biophysical processes that regulate the stability of the Earth system. This underscores the importance of achieving much greater N uptake efficiencies than currently achieved in cropping systems fertilized with highly mobile forms of synthetic N. By

coupling soil N inputs with C inputs, legumes can reduce unintentional N losses to the environment, and thus reduce the N inputs required to produce a crop (Blesh and Drinkwater, 2013; Drinkwater and Snapp, 2007).

It is unclear whether fertilized, single species stands of perennial grains would emit lower rates of the greenhouse gas nitrous oxide compared to single species stands of annual crops. Nitrous oxide fluxes are primarily driven by three interacting factors: soil nitrate concentrations, water filled pore space and soil C availability (Robertson et al., 2012). Perennial crop species are expected to reduce soil nitrate and water contents, but increase active C relative to annual crops (Robertson et al., 2011). However, pulses of soluble N fertilizer can still be vulnerable to gaseous conversion if the timing of intense precipitation events coincide with fertilization (Crews and Peoples, 2005). Since plant-available forms of legume-fixed N inputs enter through the more gradual, biological process of mineralization, the risk of nitrous oxide emissions might be expected to be relatively lower than from fertilizer sources (Jensen et al., 2012; Peoples et al., 2009b).

In addition to the economic, energetic and greenhouse gas concerns around N fertilizers, the integration of legumes into grain agriculture constitutes an important avenue of ecological intensification that can deliver pest and pathogen regulating services associated with greater crop diversity (Bommarco et al., 2013; Crews and Peoples, 2004).

## 2. Succession as an agroecosystem concept

Ecological succession is an overarching term describing directional changes in the composition of biological communities and their ecosystem processes through time (Odum, 1969). Primary succession is the process by which new rock or other soil parent materials are colonized by organisms for the first time. Secondary succession, in contrast, begins following a disturbance to an already developed ecosystem by fire, flood, drought, or any

other impact that drastically upsets the dominance of the established community (Whittaker, 1975).

Walker and Willig (1999) characterize the relative impact of terrestrial disturbance regimes based on their *frequency*, *extent* and *magnitude*. *Frequency* is the number of disturbance events per unit time, *extent* is the physical area affected by a disturbance, often reported as the proportion of a landscape affected by a disturbance event, and *magnitude* is based on the intensity and severity of the disturbance impact. Few if any terrestrial ecosystems experience the cumulative disturbance impact that characterize annual agroecosystems when frequency, extent and magnitude are considered together (Walker and Willig, 1999). Most disturbances that re-set unmanaged ecosystems to an earlier successional state or “sere” result in some loss of soil organic matter and nutrients. However, except following extreme perturbations, regrowth of vegetation commonly slows and then reverses the disturbance-related losses (Blair et al., 1998; Chapin et al., 2012). Agricultural soils that are chronically disturbed – often experiencing levels of erosion well beyond replacement rates – can be thought of as occupying a state of development somewhere between primary and secondary succession.

The concept of succession has undergone considerable scrutiny and revision in the last century (Connell and Slatyer, 1977; Götzenberger et al., 2011). No longer do ecologists see plant communities through a Clementsian lens in which highly organized coherent groupings or “superorganisms” undergo predictable additions, substitutions and losses of species through time (Clements, 1916). Communities are now seen as unique, interacting assemblages of individual species with different co-evolutionary histories. Nevertheless, community ecologists still acknowledge that directional changes in community and ecosystem properties occur, and some of these changes have relevance to agriculture. An example is the predictable, almost universal transition in plant community physiognomy from annual to perennial species following major disturbances.

### 2.1. Succession, human roles, and human failures

Crop domestication has led to remarkable increases in evolutionary success (fitness) of selected plant species. For example, wheat (*Triticum aestivum*) covers more area (220 million ha) than any other crop plant on Earth strictly because humans have found it in their interest to expand its range (Fischer et al., 2014). Similar to favoring individual species, humans have also favored certain ecosystems. In particular, we have greatly expanded the spatial extent of highly disturbed early successional seres to approximately 11% of ice-free surface area today attributable to agriculture alone. Highly disturbed, unmanaged ecosystems tend to be dominated by pioneering annual plant species, which predictably lose out to perennial species in a matter of years due to competition for light or soil resources (McLendon and Redente, 1992; Tilman, 1988).

To prepare fields for sowing annual crops, all perennial and annual vegetation covering the landscape is eliminated one or more times per year, and stocks of soil organic matter decline, regressing the ecosystem to very early secondary succession where it remains arrested for decades to centuries, until tillage ceases and perennial vegetation is restored (Smith, 2014). Work by Montgomery (2007) comparing estimates of soil formation and soil loss rates in agriculture and under native vegetation suggests that soil erosion rates exceed formation rates on a majority of agricultural landscapes—including no-till, stubble retention annual cropping systems. This begs the question of whether it is possible to build soil under annual vegetation, or whether annual agriculture is inherently dependent on soil formed under the native perennial vegetation that came before.

### 2.2. A conceptual model

To help think about agriculture in a successional context, we introduce a conceptual model in which a native grassland ecosystem is converted to annual agriculture and then allowed to return to native grassland or a perennial grain-legume intercrop (Fig. 2a–d). We term the five stages of succession depicted as: mid-steady state, retro, early-steady state, maturing, and a return to mid-steady state. We proceed to describe how a range of ecosystem properties and functions that ultimately regulate the synchrony of soil N supply and crop demand change across the successional model. In the process of considering the ecological implications of a mid-successional agriculture, we will address the following questions:

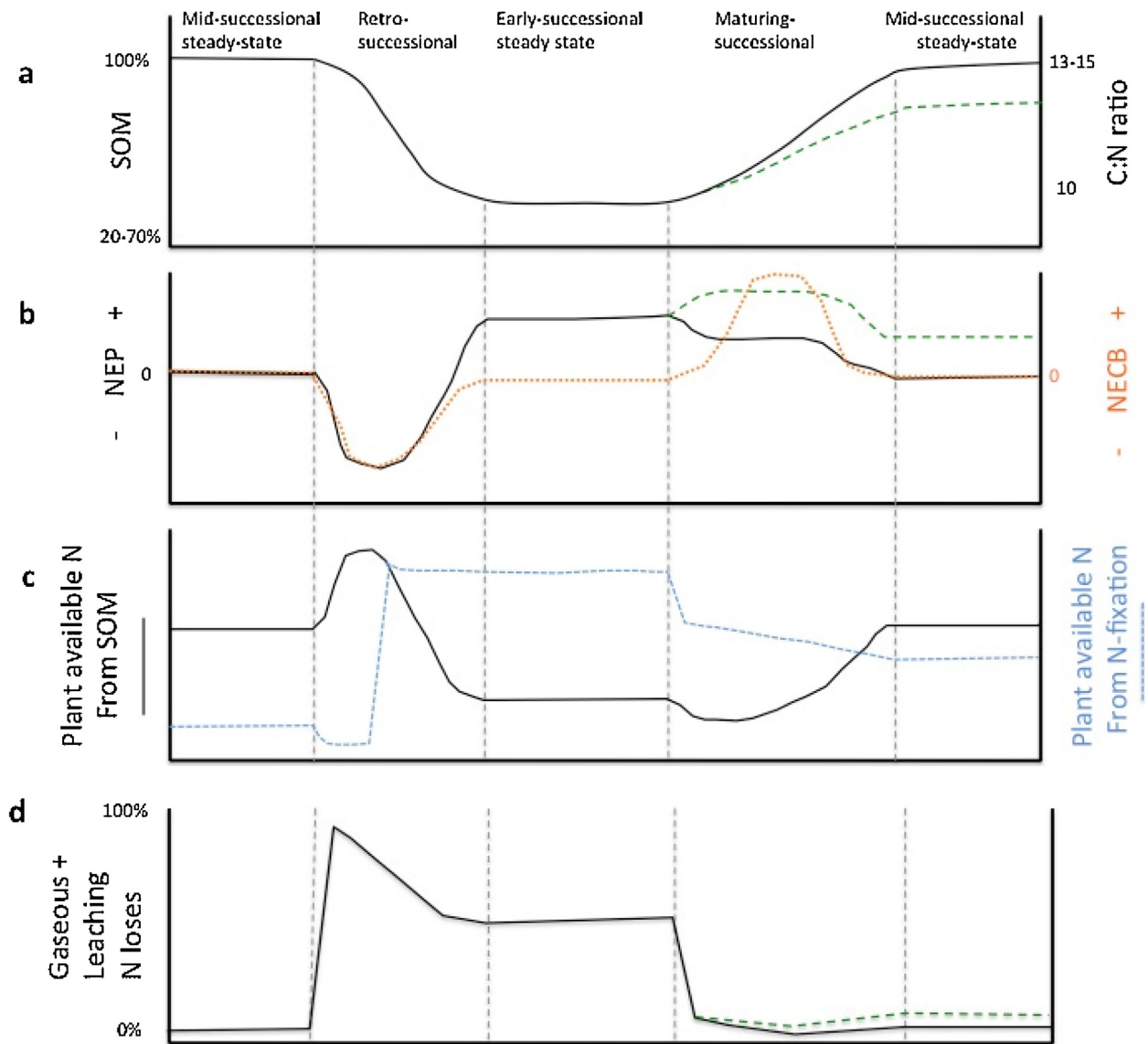
- 1) Which ecosystem functions might be expected to change as a result of a shifting from an early-successional to a mid-successional agroecosystem?
- 2) What unique challenges exist to achieving a high level of N synchrony between soil N supply and crop N demands in a perennial grain agroecosystem?
- 3) Could a mid-successional agroecosystem that features a legume-grain intercrop maintain crop productivity without inputs of synthetic N?

#### 2.2.1. Changes in soil organic matter (SOM) with ecosystem succession

Grasslands are frequently maintained in an oscillating, mid-successional steady state—sometimes functioning as a C sink during favorable climatic episodes, and sometimes as C sources, when disturbed by grazing, fire or drought, with the time-averaged C balance approaching equilibrium (Frank and Dugas, 2001; Suyker et al., 2003). The mid-successional native grassland in the simple model we propose has approached the maximum SOM content possible for a grassland ecosystem given climate, soil texture, primary productivity and disturbance regimes (Fig. 2a). When the soil is initially plowed for annual grain cropping, SOM contents immediately begin to decline (Davidson and Ackerman, 1993; Schimel et al., 1985); we call this period retro-successional as the ecosystem is set back to an earlier stage of development. SOM declines for two general reasons—increased heterotrophic respiration and lower belowground net primary productivity. With tillage, soil aggregates are pulverized and previously stabilized SOM is exposed to microbes, oxygen and warmer temperatures, resulting in rapid microbial mineralization of SOM and associated losses of organic C as CO<sub>2</sub> via microbial respiration (Lorenz and Lal, 2012). Agroecosystems tend to have less than or equal rates of net primary production (NPP) compared to the native ecosystems replaced, with the main exception of irrigated croplands (Field, 2001). Moreover, annual grains allocate approximately 15–20% of NPP belowground (Goudriaan et al., 2001; Whalen and Sampedro, 2009) in contrast to native ecosystems such as perennial grasslands that allocate closer to 50% of NPP belowground (Saugier et al., 2001).

Related to the higher belowground allocation pattern of perennials is the depth of root penetration into the soil profile. Perennial grasses have been shown to extend roots three times as deep into the profile of a sandy soil in a Mediterranean environment after only five years compared to conventional wheat and barley (*Hordeum vulgare*) crops (Ward et al., 2015). It has become increasingly clear that roots are the most important source of C inputs that feed the formation of soil organic matter (Schmidt et al., 2011). Thus, the enhanced mineralization of SOM that follows the initiation of plowing, coupled with a large reduction in SOM-forming root inputs with the shift from perennial to annual vegetation results in SOM decline as a universal feature of annual





**Fig. 2.** A conceptual model of changes in ecosystem properties that regulate the nitrogen cycle across a successional gradient. The gradient involves a well-established, mid-successional grassland that is converted to annual agriculture and then allowed to revert back to a grassland or planted to a perennial intercrop. (a) Changes in potential soil organic matter contents and C:N ratio with grassland to grassland succession (black solid line), or grassland to perennial intercrop succession (green dashed line); (b) net ecosystem productivity (NEP) with grassland to grassland succession (solid black line) or grassland to perennial intercrop succession (green dashed line) and net ecosystem carbon balance (NECB) in both grassland to grassland and grassland to perennial intercrop succession (orange dotted line); (c), plant available N from soil organic matter in both grassland to grassland and grassland to perennial intercrop succession (black solid line) and inputs of N from biological  $N_2$  fixation or the Haber-Bosch process (blue dotted line); (d) Losses of N via gaseous or leaching pathways as a percentage of N inputs with grassland to grassland succession (black solid line) or grassland to perennial intercrop succession (green dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

crop agriculture (Davidson and Ackerman, 1993; Schimel et al., 1985).

SOM declines for years to decades with continued annual cropping until a new steady state is approached between crop or crop/fallow residue inputs (or other organic matter inputs such as manure or compost) into the soil and microbially-mediated losses of C (Parton et al., 1988; Tiessen et al., 1982). By the time annual crop ecosystems have reached what we are calling the early-successional steady state, an average of 30% of SOM has been lost relative to the original native ecosystem (Davidson and Ackerman, 1993) although losses of greater than 60% have been reported (Haas et al., 1957). In some no-till systems, this steady state has

been shown to stabilize at SOM contents slightly higher than in tilled grain systems (Six et al., 2002), but these observations have been challenged recently (Powlson et al., 2014). Increasing organic inputs into soils through cover crops (Jensen et al., 2012; Poeplau and Don, 2015), legume N sources, compost or manure applications may also increase SOM contents (Drinkwater et al., 1998). But neither organic or no-till management practices will likely achieve SOM levels of the native grassland, which features both no-tillage and greater belowground organic matter inputs (Grandy and Robertson, 2007; Lorenz and Lal, 2012).

The maturing successional stage (Fig. 2a) begins with the cessation of tillage and the establishment of colonizing perennial

species. Soil organic matter contents begin to increase and will generally continue to do so for decades. Based on grassland restoration studies, a mean of  $332 \text{ kg C ha}^{-1} \text{ y}^{-1}$  with a range of  $31\text{--}1100 \text{ kg C ha}^{-1} \text{ y}^{-1}$  has been sequestered in the decades following the conversion of annual agriculture to grassland secondary succession (Post and Kwon, 2000). Perennial biofuel crops planted on historically tilled land are reported to have C sequestration rates ranging from  $160$  to  $820 \text{ kg C ha}^{-1} \text{ y}^{-1}$  (Mishra et al., 2013). The rate and duration of sequestration varies greatly depending mainly on climate, soil texture, and ecosystem productivity. Eventually, after decades to centuries, SOM contents are expected to plateau close to levels that existed before plowing (the mid-successional steady state). In some sandy or highly weathered soils, the accumulation of SOM with succession can drive down soil pH, suppress N mineralization and potentially exacerbate N limitation (Conyers et al., 2012; Helyar and Porter, 1989; Scott et al., 2000).

The conversion of an annual agroecosystem to a perennial grain-legume intercrop (as opposed to a native grassland; Fig. 2a green dashed line), would likely result in a similar increase in SOM following the cessation of tillage disturbance and the increase in belowground NPP. However, we predict that the SOM accumulated in the mid-successional steady state of the perennial agroecosystem would fall below that of the native vegetation because: (1) the introduction of an intercropped legume into the agroecosystem results in more easily decomposable and less persistent plant residues entering the soil relative to the native grassland, (2) a significant amount of NPP is removed in harvest, reducing the total SOM pool, (3) perennial grain crops may allocate less C to roots compared to native grass species, because of a shift in partitioning of dry matter from roots to seed in the breeding process, or from direct selection to reduce belowground competitiveness, (4) perennial crops would experience pulses of net SOM mineralization if tillage were used to carry out crop stand rejuvenation or replacement.

#### 2.2.2. Net ecosystem production (NEP) and net ecosystem carbon balance (NECB) through ecosystem succession

Averaged over years, the mid-successional steady state grassland has an NEP (NPP minus heterotrophic respiration) close to zero, meaning ecosystem-scale C loss in respiration approximates C gain through photosynthesis (Fig. 2b). When plowed for the first time, the retro-successional ecosystem experiences a substantial increase in microbial respiration as SOM is mineralized much faster than it is formed (Lorenz and Lal, 2012). Loss of C through enhanced microbial respiration exceeds rates of C fixed in photosynthesis or C added in other organic amendments, and the ecosystem experiences a period of negative NEP, or net C loss (negative NECB). Eventually, the rate of net mineralization slows as a new reduced SOM equilibrium is approached in the early-successional steady state. When NEP is used to describe C dynamics in most native ecosystems, all C inputs and losses are contained within the ecosystem, thus a steady state (NECB of zero) generally translates into an NEP of zero. However, in agricultural systems that export crop biomass in the form of agricultural products such as grain, hay/forage, or silage, NECB remains close to zero (the ecosystem is neither losing or gaining C on an annual basis), yet NEP remains positive—because photosynthesis of the harvested product occurs within the boundaries of the agroecosystem, but respiration of the product does not.

When annual agriculture gives way to maturing-successional grassland vegetation, NEP is expected to drop initially because harvest-NPP is no longer being exported from the ecosystem, but it remains positive due to the accumulation of SOM (Fig. 2b). This build up of SOM results in a positive NECB that is roughly the inverse of the negative NECB experienced in the retro-successional

phase. With time, SOM contents approach a maximum (Fig. 2a) and once again, respiration approximately equals NPP making NEP and NECB approach zero at mid-successional steady state.

If perennial grain-legume intercrops are planted in the maturing stage in place of native grassland vegetation, NEP is expected to become more positive than in early steady state because C exports in food continue and C accumulation begins. When the SOM maximum is approached in mid-steady state, NEP moves closer to zero. It will continue to be positive due to food exports, however it will be less positive than NEP of the early-successional steady state because food exports from the mid-steady state ecosystem would generally constitute a smaller fraction of a larger total NPP.

#### 2.2.3. Changes in plant available N with ecosystem succession

The soil C dynamics depicted in Fig. 2a–b are key for understanding controls on soil N availability in different successional stages since the microorganisms that control N cycling processes require C for maintenance and growth (Drinkwater and Snapp, 2007). Available soil N consists of all species of N that can be taken up by plants, including dissolved forms of organic N, ammonium and nitrate (Jones et al., 2005; Schimel and Bennett, 2004). In this model, the native grassland in mid-steady state maintains a relatively high rate of plant-available N entering the soil from depolymerization and mineralization of organic matter (Fig. 2c). This is supported by evidence from long-term grassland studies in which relatively large quantities of N are exported in hay crop biomass every year for decades or centuries without fertilization (Glover et al., 2010; Johnston et al., 2009). We discuss possible mechanisms explaining how high fluxes of N through plant available N pools might be maintained in the mid-successional steady state in Section 3.

The flush of SOM mineralization after tillage in the retro-successional stage results in a net release of mineral N and other nutrients liberated from organic bonds (Vitousek et al., 1989). Agriculturalists throughout the ages have cropped intensively to take advantage of this pulse of nutrients that ensues after plowing when NEP is negative (Mazoyer and Roudart, 2006; Robertson, 1997). In many cases net N mineralization rates can exceed crop uptake particularly early in the crop's development and after harvest, when N-demand is low, leaving N vulnerable to loss via leaching or denitrification. As the SOM pool approaches the early-successional steady state and NEP becomes positive, net N mineralization declines. A persistent state of positive NEP, in this case maintained with annual cropping, will generally result in a condition of chronic N limitation (productivity runs ahead of N mineralization from SOM), at least with respect to SOM as an N source. Reduced available N from SOM in the early steady state necessitates greater N inputs through biological  $\text{N}_2$  fixation or synthetic fertilizers (blue dotted line, Fig. 2c) to balance intentional (harvest) and unintentional (leaching and gaseous) losses from the annual crop ecosystem.

Plant available N is predicted to decline initially in the transition from annual grain to the mid-successional grassland ecosystem, as N is immobilized in a stoichiometric ratio with C in the aggrading SOM pool (Kirkby et al., 2011). For example, if an early maturing grassland sequesters the average  $332 \text{ kg C ha}^{-1} \text{ y}^{-1}$  reported by Post and Kwon (2000), then somewhere between 22 and 33 kg N  $\text{ha}^{-1} \text{ y}^{-1}$  will be sequestered as well, based on the C:N ratio of most SOM which ranges between 10 and 15:1. As the SOM pool builds, however, the ecosystem begins to accumulate and cycle more organically bound N, which eventually increases N availability until mid-successional steady state is reached.

During a transition from annual grain to a mid-successional grain agroecosystem, inputs of N from  $\text{N}_2$  fixation need to be maintained above harvest-N replacement because N is also being

“lost” from the ecosystem in the aggrading SOM pool. That said, the fixed N input needed to sustain harvested N exports is expected to decline through the maturing stage as the expansion of the SOM pool slows, less N is immobilized, and the N supplying capacity of the agroecosystem is increased (Drinkwater and Snapp 2007). In practice, the decline occurs either because of lower legume planting densities required to meet crop N demand, or lower legume reliance on  $N_2$  fixation for growth as the result of higher levels of available N in soil. Given the need to harvest grain, the mid-successional steady state stage of a perennial grain-legume intercrop will always maintain a higher throughput of N than the tight-cycling N economy of the native prairie. Over time, the N budget of the intercrop can only be balanced if biological  $N_2$  fixation by legumes, soil crusts, plant endophytes and N inputs from other sources equal crop-N exports. Compared to annual agroecosystems, perennial grain ecosystems will require less fixed N to compensate for unintentional losses. For example, the amount of N lost by leaching in a comparison of annual wheat and an established perennial wheatgrass field was 27.5 and 0.5 kg ha<sup>-1</sup>, respectively (Culman et al., 2013). This translates into 27 kg ha<sup>-1</sup> yr<sup>-1</sup> less N input required to balance the N-budget in the perennial system.

#### 2.2.4. Changes in N losses via gaseous and leaching pathways

Vitousek and Reiners (1975) proposed a conceptual model that predicted a high degree of nutrient retention in mid-successional ecosystems, and low retention in disturbed, early successional systems. Numerous studies of native grasslands and forested ecosystems have supported this model (e.g., Dodds et al., 1996; Likens et al., 1977). In Fig. 2d, the native grassland experiences very low levels of N loss consistent with relatively low N inputs and an NEP close to zero. When the grassland system is plowed for agriculture, N losses reach a maximum as N released from SOM mineralization exceeds requirements by annual crops. Once the early-successional steady state is reached, losses of N might represent about half of inputs (through fertilizer) due to poor synchrony between N supplies and crop N demand (Crews and Peoples, 2005; Ladha et al., 2005; Robertson et al., 2012). In the maturing-successional phase, native vegetation or perennial intercrops experience reduced N losses because more soil N is held in less mobile organic forms, perennial roots take up more N in both time and space, and aggrading SOM pools provide an additional C sink for mineral N resources.

A growing body of research overwhelmingly supports the prediction that perennial agroecosystems experience lower N leaching losses than annual systems. For example, Syswerda et al. (2012) reported average nitrate leaching losses ranging from 19 to 62 kg ha<sup>-1</sup> yr<sup>-1</sup> compared to 0.01–12.9 kg ha<sup>-1</sup> yr<sup>-1</sup> for annual and perennial crops, respectively. In a study comparing annual and perennial crops for bioenergy production in Denmark, Pugesgaard et al. (2015) measured leaching losses of 14.3 kg NO<sub>3</sub>-N ha<sup>-1</sup> over three years, or 2.1% of the 687 kg of N ha<sup>-1</sup> applied, at 1 m below a fertilized perennial grass-clover treatment. Additional N inputs from biological N fixation to the grass-clover system were estimated at 163 kg ha<sup>-1</sup> over three years. In contrast, 162.8 kg NO<sub>3</sub>-N ha<sup>-1</sup>, or 31.4% of total N applied during the same period was leached to 1 m depth under stands of winter wheat.

Over time, as the perennial intercrop approaches steady-state conditions, N losses and N uptake efficiency will be driven by differences between N inputs and harvested N exports (Aber et al., 1989; Fenn et al., 1998; Vitousek, 2004). Despite evidence for improved efficiencies of perennial compared to annual ecosystems, N saturation and N mass balance frameworks indicate that all ecosystems can lose N under continual high N inputs that exceed a given ecosystem's capacity to immobilize and retain N through

plant and microbial sinks. Conditions that favor N leaching are less likely to occur in legume-based intercrops, but leaching could easily occur with excessive applications of synthetic N, manures, or composts. For example, in perennial turfgrass (Carey et al., 2012) as well as perennial grain crops (Culman et al., 2013), leaching losses of nitrate have been found to increase with greater rates of inorganic N fertilizer.

### 3. Mechanisms governing N availability across successional stages

The largest pool of N that directly supplies wild and domesticated plants across the successional gradient is SOM. This is a proximate source, with the ultimate sources being biotic and abiotic  $N_2$  fixation. But proximate matters, because the relative rates of N mineralization and immobilization largely determine the degree of N synchrony that is achievable in a particular season—too little mineralization and the vegetation encounters N limitation; too much mineralization and N is leached or lost in gaseous forms from the ecosystem. A wide range of studies including cultivated and native ecosystems suggest that between 0.5 and 6% of total N contained in SOM undergoes net mineralization annually (Cassman et al., 2002; Millar and Robertson, 2015).

Temperature and moisture conditions can contribute to the wide variation in microbial activities responsible for N mineralization. For example, precipitation events that follow long periods of drought have been shown to trigger specific populations of microbes resulting in large pulses of SOM mineralization and release of available N (Placella and Firestone, 2013). The very presence of perennial species or particular plant functional groups can impact soil microbial populations and influence decomposition dynamics. In a comparison of perennial C<sub>4</sub> grasses with wheat and barley, Gupta et al. (2014) demonstrated faster rates of decomposition under perennial grasses, which they attributed to dominance of copiotrophic bacterial communities (e.g., proteobacteria), and more active microbial communities in general. The abundance of legumes in particular can enhance root decomposition and N release (Fornara et al., 2009). Soil organic matter quality and quantity (e.g., the particulate organic matter to SOM ratio, Marriott and Wander, 2006) and disturbance through tillage also play significant roles in determining SOM mineralization rates.

How these mechanisms governing N availability vary across successional states is illustrated by data from the Main Cropping System Experiment (MCSE) at the Kellogg Long Term Ecological Research Site in Michigan (Table 1) (Millar and Robertson, 2015). The mown grassland treatment of MCSE was never plowed or fertilized, and was mowed every fall, with harvested grass left in place to decompose. It most closely corresponds to the mid-successional steady state in Fig. 2. Being the only never-plowed treatment at MCSE, it maintained the highest total soil N, but it experienced the lowest net N mineralization on an absolute and percentage of total-N basis (Table 1). This low N mineralization was likely due in part to this grassland containing very low legume abundance, which can contribute to low levels of labile organic N, and partly due to the lack of disturbance through tillage. In contrast, the perennial legume alfalfa (lucerne; *Medicago sativa*) maintained high total-N and the highest net N mineralization of any annual or perennial treatment at 192 kg ha<sup>-1</sup> season<sup>-1</sup> (Table 1).

The effects of tillage on the mown grassland were demonstrated when subplots of the mid-successional grassland were plowed. Within 30 days, net mineralization of SOM spiked, resulting in a doubling of CO<sub>2</sub> fluxes from microbial respiration, and concomitant increases in soil ammonium, nitrate and nitrous oxide fluxes (Grandy and Robertson, 2006). This pattern is consistent with predicted changes in available N in the retro-successional stage of the conceptual model (Fig. 2a).

**Table 1**

Mean total soil N content, net N mineralization rates and % of total N mineralized per year in the A/Ap horizon of the Kellogg Biological Station Main Cropping System Experiment (MCSE) treatments (Millar and Robertson 2015).

System	Total N (kg ha <sup>-1</sup> )	Net N Min Rate (kg ha <sup>-1</sup> season <sup>-1</sup> )	Net N Min as % of Total N
<b>Annual Cropping Systems</b>			
Conventional <sup>a</sup>	3580	99	2.8
No-till <sup>b</sup>	3630	113	3.1
Reduced input <sup>c</sup>	3720	178	4.8
Biologically based <sup>d</sup>	3510	163	4.6
<b>Perennial Crop &amp; Successional Communities</b>			
Alfalfa <sup>e</sup>	4050	192	4.7
Early <sup>f</sup>	3900	90	2.3
Mown grassland <sup>g</sup>	5950	27	0.5
Mid-successional <sup>h</sup>	4080	113	2.8

<sup>a</sup> Conventional: corn-soybean-winter wheat rotation, standard chemical inputs, chisel-plowed, no cover crops, no mature or compost.

<sup>b</sup> No-till: corn-soybean-winter wheat rotation, standard chemical inputs, permanent no-till, no cover crops, no manure or compost.

<sup>c</sup> Reduced Input: corn-soybean-winter wheat rotation managed to reduce synthetic chemical inputs, chisel-plowed, red clover or annual rye winter cover crop, no manure or compost.

<sup>d</sup> Biologically Based: corn-soybean-winter wheat rotation, no synthetic chemical inputs, chisel-plowed, mechanical weed control, red clover or annual winter cover crop, no manure or compost, certified organic.

<sup>e</sup> Alfalfa: 5–6 year rotation with winter wheat as a 1-year break crop.

<sup>f</sup> Early: successional community, historically tilled cropland abandoned in 1988, burned every year in spring to prevent woody colonization of grassland.

<sup>g</sup> Mown grassland: successional community, cleared woodlot in the 1950s, never tilled, mowed in the fall to prevent woody establishment in grassland.

<sup>h</sup> Mid-successional: historically tilled cropland abandoned ca. 1955, no mowing or burning, regrowth in transition to forest.

The MCSE annual cropping systems in Table 1 represent the early-successional steady state. Net mineralization was lowest in the conventional system that experiences annual tillage with no cover crop. Net mineralization was greater in the reduced input and biologically based systems, likely because of additional organic matter added to the system via cover crops, introducing N-rich residue that supports an active, more readily mineralized SOM pool (Paul et al., 2015).

When a cultivated system is abandoned and the ecosystem enters the maturing succession stage (Fig. 2a), net N mineralization is predicted to decrease due to N sequestration in aggrading SOM. The early successional MCSE plots reflect this pattern, with the total-N pool aggrading, but net N mineralization decreasing as a percentage of total N (Table 1). While the MCSE plots do not include a legume-rich successional treatment, it is interesting to consider what the N mineralization rates might be in such a treatment. Based on the data reported for alfalfa (Table 1), a perennial legume-grain bi-culture has the potential to achieve greater rates of net N mineralization than the 90 kg ha<sup>-1</sup> measured in the early successional plots. Other MCSE treatments that include legumes in the cropping system appear to maintain greater net N mineralization rates on an absolute basis and as a percentage of total soil N (Millar and Robertson, 2015; Paul et al., 2015). Annual cropping systems have approximately the same total-N, and yet the two systems with legume-rye (*Secale cereale*) cover crops—reduced input and biologically based—maintain higher N mineralization rates (Table 1). The role of the legume is also apparent in comparing N mineralization in the alfalfa treatment, which averaged 192 kg ha<sup>-1</sup> season<sup>-1</sup> with the early successional and mown grassland (never tilled) treatments, averaging 90 and 27 kg ha<sup>-1</sup> season<sup>-1</sup>, respectively.

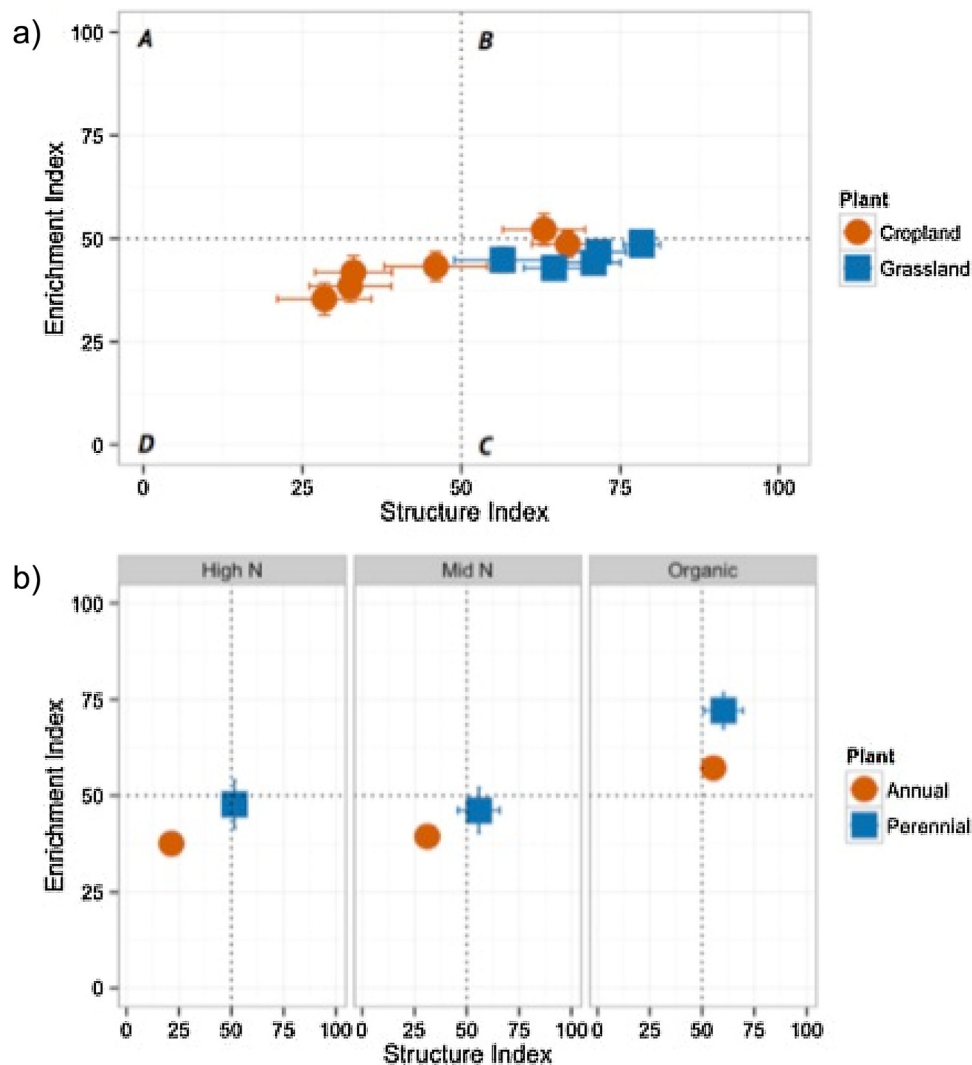
### 3.1. Changes in soil communities that influence N cycling during succession

The succession of plant communities in terrestrial ecosystems is typically accompanied by shifts in belowground microbial communities and subsequent changes in ecosystem functions (Bardgett and Wardle, 2003; van der Heijden et al., 2008; Wardle, 2004). The collective soil food web, including bacteria, fungi, nematodes, micro- and macro- arthropods, is strongly shaped by plant inputs (Wardle, 2004), and reciprocally influences plant community composition by mediating decomposition and mineralization processes (Fornara et al., 2009; Holtkamp et al., 2011; Kardol et al., 2006; Koziol and Bever, 2015; Wickings et al., 2012). The predominate factors driving food web changes with ecosystem succession are shifts in resource availability and in microhabitats including soil pH (Maharning et al., 2009). As plant community composition shifts during succession, the quality and quantity of plant residues entering decomposition channels also change. Nitrogen mineralization rates typically increase with greater soil microbial biomass during succession (Holtkamp et al., 2011), but they tend to diminish with plant litter chemistry characterized by higher C to nutrient ratios (Williamson et al., 2005) and increases in fungal: bacterial dominated decomposition channels (Bardgett et al., 1996; Williamson et al., 2005).

Only a limited amount of research is available to shed light on overarching changes in the belowground community with succession, but recently Bardgett and van der Putten (2014) suggested that some broad patterns are emerging. They proposed that early in succession, soil food webs are dominated by simple, heterotrophic microbial communities as well as photosynthetic and N<sub>2</sub>-fixing bacteria. As succession progresses, the belowground communities become more complex and stable, characterized by increasing food chain length, a reduced role of soil pathogens and greater reliance on mycorrhizal fungi for plant nutrition. A large number of belowground effects have been documented through ecological succession, but the interactions of N cycling and the soil food web are of specific interest here. Because N availability is often a constraint to primary production in terrestrial ecosystems (Vitousek and Howarth 1991), and tends to be even more limiting in agricultural systems, which have regular N exports in harvests, we will review findings from two field studies that illustrate the possible roles of soil food webs in enhancing N availability in mid-succession, perennial systems.

Research in Kansas, USA examined soil food webs under harvested native perennial grasslands (i.e., tallgrass prairie) and annual wheat (Culman et al., 2010). The grasslands had been harvested without fertilization for 75 years, yet still remained productive and maintained much greater levels of soil C, N and aggregation than adjacent wheat fields. Examination of soil biota showed large differences between the two systems. Food web indices based on nematode community composition (Ferris et al., 2001), revealed that soil biota in the harvested grasslands were more structured and diverse, but maintained equal levels of nutrient cycling compared with wheat (Fig. 3a; adapted from Culman et al., 2010). Soil food web complexity closely tracked root biomass, with diminished food webs at the lower depths under annual wheat, but not under the perennial grasslands. These differences highlight the intimate role that plant root C inputs play in maintaining belowground community structure, influencing the functioning of ecosystems, such as pest suppression and energy and nutrient flows (Bardgett and Wardle, 2003). For example, the calculated N mineralization rate attributable to nematode communities in this particular study was estimated at 28 kg N ha<sup>-1</sup> yr<sup>-1</sup> in grassland soils, compared to an estimated 16 kg N ha<sup>-1</sup> yr<sup>-1</sup> in cropland soils (Culman et al., 2010). As nematodes typically mineralize only a fraction of the total N mineralized by the entire





**Fig. 3.** (a and b) Soil food web indices based on nematode communities yield insight into food web dynamics, such as rates of nutrient cycling (enrichment index) or food web complexity (structure index). Top panel (a) soil food webs from harvested perennial grasslands (squares) demonstrated equal level of nutrient cycling (i.e., enrichment index) as annual wheat (circles). Grassland food webs were more complex (i.e., structure index) than food webs in wheat, especially at lower depths in the soil profile. Bottom panel (b) illustrates more enriched and complex food webs under a perennial grain system compared to annual wheat over three levels of N availability. Typical agricultural systems exhibit food webs in Quadrant A (high nutrient cycling, low structure), while typical non-harvested natural systems are found in Quadrant C (low nutrient cycling, high structure). Intensively-managed perennial grain systems may be unique having food webs occupy Quadrant B (high nutrient cycling and high structure).

soil food web (Schroter et al., 2003), the whole soil biota may play a pivotal role in synchronizing rates of N availability in the examined perennial grasslands.

Similar trends were observed in a cropping system trial in Michigan, USA consisting of Kernza<sup>®</sup> perennial grain wheatgrass (*Thinopyrum intermedium*) and annual wheat under three N fertilization regimes: high N (urea), low N (urea), and organic (chicken manure). Four years after establishment, soil food webs under the perennial grain system were more complex and enriched than under wheat in all three fertilization regimes (Fig. 3b; Culman et al., unpublished data). Soil food webs in the organic system, which received the lowest rates of available N, showed greater amounts of N moving through the food web (i.e., greater enrichment index) than treatments fertilized with mineral N.

### 3.2. Nitrogen translocation in perennials

The mineralization of N is the most important proximate process that sustains the supply of available N to wild plants and crops across the successional gradient. The ability for plant species to take up N and store it until N demand is high relative to soil N availability is important for improving synchrony. Translocation could be a key mechanism to meet periods of high N demand such as during seed filling, or strictly in the case of perennials, emergence and regrowth after senescence. Perennials translocate N to roots and crowns before dormant periods. During foliage senescence, approximately half the remaining aboveground N is recycled to support new growth belowground (Chapin et al., 1990). This recycled N is primarily converted from proteins to amino acids and translocated to roots and crowns that persist through the dormant season (Hayes, 1985).

Internally translocated N represents a substantial N flow in perennial vegetation that can support rapid, early season growth. During a full growing season, the mobilization of stored N may support as much as 50% of aboveground N across a variety of perennial grassland, bioenergy and forage grasses (Bausenwein et al., 2001; Clark, 1977; Strullu et al., 2011). The amount of N remobilized to support spring growth is linearly related to plant N reserves (Strullu et al., 2011). Biomass harvests prior to senescence, or conditions of limited soil N availability, can reduce the N available for translocation to roots and crowns and, therefore, N available for spring growth (Strullu et al., 2011). During reproduction, resources are primarily translocated from existing leaves to support seed production (Chapin et al., 1990). In perennial grains, there may be a trade-off between grain harvest and the development of N reserves to support the following year's growth.

Perennials rely more on internal N reserves during early growth following dormancy when carbohydrate reserves are low. For example, Bausenwein et al. (2001) found that internally translocated N contributed more than 70% of the N in new spring growth over more than 90 days in two perennial grasses. Translocated N can also support rapid regrowth following defoliation events (Ourry et al., 1990, 1994). Nitrogen stored in crowns and older leaves provides the most mobile source of N for regrowth (Bausenwein et al., 2001; Santos et al., 2002). To illustrate the relative mobility of internally translocated N, the N in roots and crowns in permanent grasslands at Rothamsted in the United Kingdom, had an estimated turnover time of 0.4–1.4 years (Jenkinson et al., 2004). This was a faster turnover rate than N in microbial biomass (~2 years) or background SOM (~150 years). A minimum threshold exists for the amount of N that must remain in root and crown biomass to maintain basic metabolic and structural N requirements. For the bioenergy crop, *Miscanthus giganteus*, the threshold was approximately 50 kg N ha<sup>-1</sup> that was not available for translocation to new growth (Strullu et al., 2011).

Reliance on internal N translocation in herbaceous perennial species can also vary depending on available resources, such as N and water. Under conditions of N and water stress, perennials can increase the proportional reliance on N mobilization to new growth both above- and belowground (Hayes, 1985; Li et al., 1992). This suggests that internal translocation provides an important mechanism for maintaining productivity in perennial systems under variable environmental conditions.

The relative allocation of N to different plant organs differs among perennial species and cultivars. Perennials adapted to N-limited environments, for example, often allocate more N to belowground organs to conserve limited resources (Chapin et al., 1990; Louahlia et al., 2000). Under N-limited conditions, some species reduce N allocation to new tillers and preferentially translocate it toward leaf growth. In contrast, other species show less plasticity in response to N availability and maintain similar relative allocation to tillers, roots, and new leaf growth (Santos et al., 2002). To support production and maintain N cycling efficiency across the multiple stages of ecosystem development in the transition from an annual to a perennial grain-based agriculture, it may be important to retain some plasticity in N allocation patterns of perennial grain crops.

#### 4. Nitrogen limitation under conditions of positive NEP and the role of legumes

The degree of N limitation that a native, mid-successional plant community experiences corresponds to the synchrony between the N required to achieve potential productivity, and the N actually mineralized from microbial decomposition of soil SOM. Differences in the C:N of SOM and microorganisms links microbial respiration of CO<sub>2</sub> with the release of mineral N (ammonification).

Short-term asynchronies between plant productivity and N release from microbial activities can be attenuated or overcome with adaptations like retranslocation of N or the scavenging of soil N by roots and hyphae of mycorrhizal symbioses (Johansen et al., 1993, 1994). However, if NPP exceeds microbial respiration across multiple growing seasons (persistently positive NEP) then persistent N limitation develops as N demands for plant growth run ahead of N supply, unless N inputs are enhanced by N<sub>2</sub>-fixing organisms or via provision of an external N source.

There are only a few stages in native ecosystem succession when NEP remains positive for extended periods of time. The first is primary succession, when vegetation is developing with initially restricted available soil N resources. The combination of reduced available N due to N immobilization in an accruing SOM pool, coupled with greater N demand from increasing NPP results in N limitation (Vitousek and Farrington, 1997). Legume-rhizobia symbioses, and N<sub>2</sub>-fixing actinorhizal plant symbioses such as alder (*Alnus* spp.) trees and *Frankia* spp. are widely recognized for their roles in providing N to make C acquisition possible during the positive NEP period of primary succession (Vitousek and Howarth, 1991).

As a native ecosystem approaches full stature and achieves its maximum rate of NPP, then growth in N demand also plateaus, and SOM mineralization supplies more and more of the vegetation's N requirements, shifting the role of biological and inorganic N fixation from a critical input required to support increases in NPP, to more of a "topping off" function, to compensate for the N lost through leaching, runoff or gaseous emissions. This topping off may be supplied by legume-rhizobia or actinorhizal plant-bacteria symbioses, but might also be supplied by free-living N<sub>2</sub>-fixing bacteria (Reed et al., 2011), or atmospheric deposition of N in rainfall or dust. Ecosystems in this stage reflect the N economy of the mid-successional equilibrium. The retro-successional stage following disturbance by tillage results in negative NEP—respiration exceeds NPP. In this case, N mineralization exceeds vegetation requirements making net contributions of legume N<sub>2</sub> fixation to the soil, or other exogenous N inputs unnecessary for a period of time.

The second example of when native ecosystems commonly experience extended periods of positive NEP is in early to mid-secondary succession. It occurs when the disturbance that initiates secondary succession is intense enough to induce a significant pulse of SOM mineralization that results in C and N loss from the ecosystem. As in primary succession described above, the maturing successional stage that follows will be characterized by N limitation as the aggrading SOM pool is immobilizing N at the same time NPP is recovering toward the ecosystem maximum. This describes the economy of the maturing successional stage (Fig. 2a). Symbiotic and asymbiotic N<sub>2</sub>-fixing organisms can play significant roles in meeting the N deficit inherent in protracted positive NEP.

The difference between annual agriculture and secondary succession in native ecosystems is the former experiences ongoing frequent disturbance through tillage or herbicides and periods of fallow between crops that preclude even the earliest successional changes that improve ecosystem functions such as nutrient retention (Vitousek and Reiners, 1975). Thus annual grain cropping systems experience progressively acute and chronic N limitation because: (1) the export of N-rich grain biomass perpetuates a state of positive NEP, since productivity exceeds respiration, (2) losses of SOM, and (3) significant quantities of reactive N are lost from the ecosystem during disturbances such as tillage or bare fallow periods when plant roots are absent or senesced. To balance the N budget of the annual grain ecosystem, prodigious inputs of reactive N are required. Historically, legumes included in crop rotations have played a prominent role in providing this N (Angus et al., 2015), although the Haber-Bosch industrial process for

synthesizing ammonia has superseded legumes as the most important N input to global agriculture in the late 20th century (Smil, 2001). In the following section we consider how legumes might function to help sustain the N economy of perennial compared to annual cropping systems.

#### 4.1. Legume N<sub>2</sub> fixation in annual versus perennial systems

Biological N<sub>2</sub> fixation by some diazotrophs can occur in a free-living state, and via associative or symbiotic relationships with plants. Symbiotic associations between legumes and soil bacteria (collectively rhizobia) in specialized root structures (nodules) are responsible for the largest inputs of biologically fixed N in agricultural systems (Herridge et al., 2008). Contributions of fixed N by legumes, whether annual or perennial, are subject to many genetic variables and influenced by a diverse range of biotic and abiotic constraints (Peoples et al., 2009a, 2012), but ultimately the amount of N<sub>2</sub> fixed reflects two key factors: (i) the amount of legume N accumulated over the growing season (usually determined by shoot dry matter [DM] production and %N content), and (ii) the proportion of the legume N derived from atmospheric N<sub>2</sub> (often abbreviated as %Nd<sub>f</sub>a):

Amount of legume

$$\text{shoot N fixed} = \left( \frac{\text{legume shoot DM} \times \%N}{100} \right) \times \left( \frac{\%Nd_{f}a}{100} \right)$$

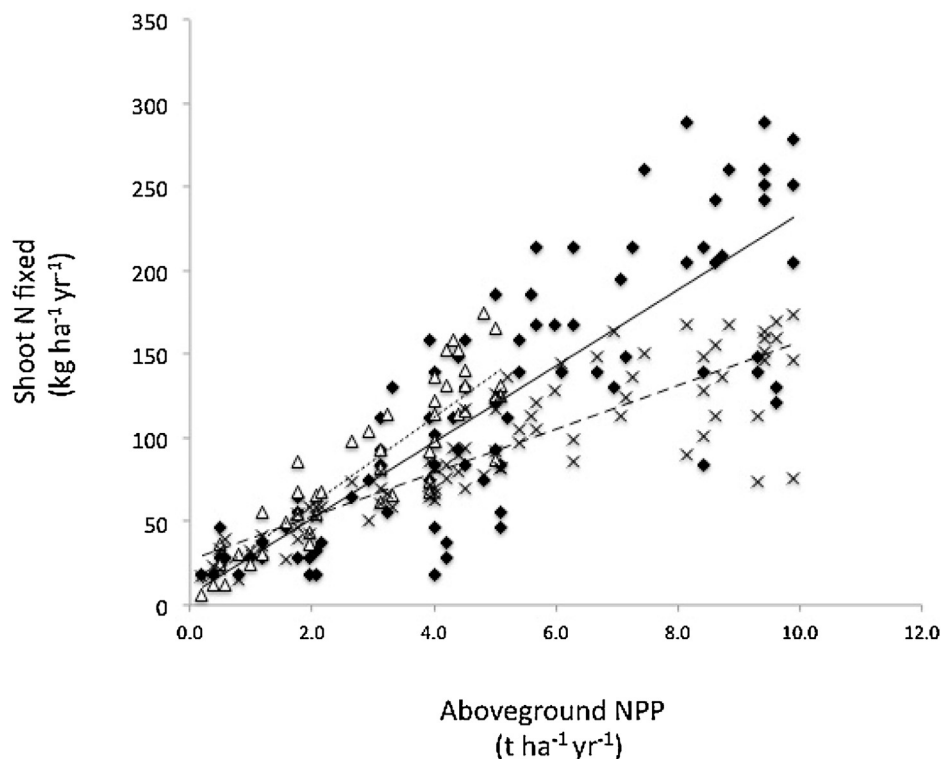
Although the levels of %Nd<sub>f</sub>a are important, provided there are adequate numbers of effective rhizobia in the soil, and concentrations of soil mineral N are not so high as to delay nodulation or suppress the N<sub>2</sub> fixation process (i.e., >75 kg N ha<sup>-1</sup>), the amounts of N<sub>2</sub> fixed will be overwhelmingly regulated by legume growth and total biomass production rather than by %Nd<sub>f</sub>a (Fig. 4). Many

legume crops and forages tend to fix between 15 and 25 kg shoot N for every tonne (t; 1000 kg) of shoot DM accumulated, with an average commonly around 20 kg shoot N t shoot DM<sup>-1</sup> (Anglade et al., 2015; Carlsson and Huss-Danell, 2003; Peoples et al., 2012; Unkovich et al., 2010). Higher amounts of fixed N per unit productivity in forage species (Fig. 4) is likely due to (a) shoots of forage species tending to have a higher ratio of leaves to stems and thus maintaining higher %N content than crop legumes; and (b), legumes in pastures are often grown in mixtures with grasses or other non-legume species whose roots are more competitive than legumes in capturing soil mineral N, thus driving greater dependence on N<sub>2</sub> fixation by the legumes (Dear et al., 1999). These factors and other characteristics of perennials such as longer effective growing seasons result in some perennial forage legumes contributing higher inputs of fixed N to agroecosystems than annual forage or crop legumes (Fig. 4; Peoples et al., 2012).

It is common to inoculate annual legumes with highly efficient rhizobia symbionts at the time of sowing. In perennial agroecosystems, there is limited opportunity to inoculate stands with efficient rhizobia beyond the establishment year, regardless of whether the legumes are perennial or self-re-seeding annuals. The background rhizobia community that develops over time in the perennial agroecosystems will likely be diverse, with wide variation in N-fixing efficiencies (Ballard and Charman, 2000; Drew et al., 2012). This could present a unique challenge, one that might be addressed by breeding for legume genotypes that impose sanctions on inefficient rhizobia symbionts (Kiers et al., 2003).

##### 4.1.1. Factors that regulate legume productivity

Given the critical role of legume productivity in determining total inputs of N<sub>2</sub> fixation, it is useful to consider several key factors that regulate legume productivity and how they differ in annual versus perennial intercrops. Competition for light, water and



**Fig. 4.** The amounts of shoot N fixed as a function of aboveground net primary productivity (ANPP) in perennial forage legumes (solid diamonds,  $n = 83$ ,  $y = 22.81x + 6.21$ ,  $R^2 = 0.68$ ), annual forage legumes (open triangles,  $n = 46$ ,  $y = 26.01x + 8.37$ ,  $R^2 = 0.78$ ), and annual crop legumes (Xs,  $n = 123$ ,  $y = 13.06 + 26.92$ ,  $R^2 = 0.75$ ). Perennial forage data from Carlsson and Huss-Danell (2003), annual forage data from Unkovich et al. (2010), and annual crop legume data from Peoples et al. (2009a).

nutrients (particularly N) are among the most significant interactions that regulate productivity of intercropped legumes. Soussana and Tallec (2010) reviewed experimental evidence suggesting that  $N_2$  fixation by legumes was tightly coupled to N demand by associated grasses via physiological, morphological and ecosystem-scale mechanisms in grasslands. They further developed this coupling with a model that showed biological  $N_2$  fixation in legumes increasing when the N status of grasses declines; presumably reflecting low available soil N. Declining N status is also commonly associated with reduced growth and vigor resulting in less competitive pressure (for water and non-N nutrients) from the grass on the legume, which in turn contributes to enhanced legume growth and  $N_2$  fixation (Ledgard and Steele, 1992). Soussana and Tallec (2010) concluded that the grass-clover mixture model would tend toward a homeostasis under constant environmental conditions. However, observations in actual grass-legume mixtures such as in perennial pasture settings demonstrate how variation in amount or timing of precipitation or herbivory, or other environmental factors can disrupt grass-legume equilibrium, and result in dominance of one species, or cycles of legume-grass dominance.

In both annual and perennial systems, competition can be managed through careful selection and even breeding of compatible species. Annual systems have the advantage in that crop spacing can be determined with planting, and this spacing can be re-set every year with re-planting. In perennial grass-legume intercrops, individual plants increase in size over time and many can reproduce asexually spreading through rhizomes or tillers. Manipulation of the grain and/or legume crop by selective cutting, grazing or burning and overseeding of the legume can shift the competitive balance between the species, leading to a dynamic equilibrium in which the intercropped species alternate in dominance over the course of one or more years (Fig. 1). While there are some very useful design and management approaches to work with, interspecific competition may prove to be the most challenging factor limiting legume productivity and ultimately N supply to the grain crop in an intercrop arrangement (Vandermeer, 1989). In some ecosystems, N-inputs for perennial grains may best be supported with intercropped, self-seeding annual legumes that better partition resources of water and sunlight.

#### 4.1.2. Nutrients other than N

Soil P availability is a factor that can strongly influence rates of  $N_2$  fixation (Crews, 1993; Vitousek et al., 2010) and legume productivity, and thus affects total fixed N entering the grain-legume intercrop (Peoples et al., 2012; Soussana and Tallec, 2010). Interestingly, Houlton et al. (2008) hypothesized that legumes evolved the capacity to fix N in order to access and more quickly cycle forms of soil organic-P, by producing greater quantities of N-rich, extracellular phosphatase enzymes and by acidifying the rhizosphere via exudation of  $H^+$  (Hinsinger et al., 2011). While both annual and perennial legumes are capable of producing phosphatase enzymes, the concentrations of accessible organic-P are typically much greater in mid-successional soils conditioned by perennial vegetation compared to early-successional soils that support annual crops (Crews and Brookes, 2014; Daroub et al., 2001). Thus perennial legumes occupying mid-successional soils may benefit from the organic P pool in addition to the inorganic P pools that constitute the primary sources of P in annual crop agriculture. Furthermore, the roots of some legumes release organic acids to solubilize and access otherwise unavailable forms of P (Angus et al., 2015), so intercropped perennial grain crops could also potentially gain a P benefit from the correct choice of legume partner. Sulfur (S) is another essential element that exists both in inorganic and organic forms in the soil (Lucheta and Lambais, 2012). Similar to P, the larger pools of organic-S in mid-

successional compared to early-successional soils may also increase the S-nutrition and productivity of legumes.

Low availability of other lithophilic or rock-derived nutrients other than P and S can also limit legume  $N_2$  fixation (O'Hara, 2001; Peoples et al., 2012), but lacking distinct organic forms in soils, they have similar availability under annual and perennial vegetation. However, some perennial root systems can access nutrients deeper in the soil profile, which could improve legume productivity and total  $N_2$  fixation.

#### 4.1.3. Soil mineral N

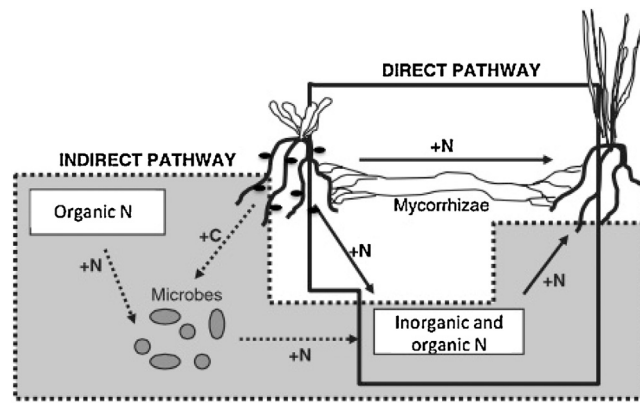
When levels of soil mineral N are elevated, legumes rely to a greater extent on root uptake of N since  $N_2$  fixation is an energetically "expensive" process, and will subsequently reduce their reliance upon  $N_2$  fixation for growth (Peoples et al., 2009a, 2012). Most reports focus on nitrate as the form of N that most commonly reduces %Ndff, however ammonium has also been found to suppress  $N_2$  fixation in some legumes (Peoples et al., 1989). One way that farmers have been able to maintain high % Ndff in annual cropping systems, is to plant legume cover crops into soils following heavy feeding non-legumes, which depletes soil mineral N pools. This strategy is not possible in a perennial system. Alternatively, nitrate tends to be depleted by non-legume components of annual intercrop systems inducing higher %Ndff by the legumes (Bedoussac et al., 2015). However, while individual legume plants may fix a higher percentage of their N requirements when grown in association with a non-legume, monocultures of legumes commonly fix more per unit area because of greater legume biomass production (Bedoussac et al., 2015).

The %Ndff of perennial legumes can also be influenced by soil nitrate concentrations (Carlsson and Huss-Danell, 2003; Peoples et al., 2012), and intercropped perennial grasses have been found to raise legume %Ndff by outcompeting the legume for soil N resources (e.g., Dear et al., 1999; Jørgensen et al., 1999). Mid-successional soils arrived at and maintained by perennial species are less likely to experience the degree of N mineralization that can result in suppression of  $N_2$  fixation that occurs more commonly under annual tilled conditions. Thus perennial-grain intercrops may rarely be challenged by mineral N suppression of N fixation, especially when N stocks are depleted by the annual harvests of high protein seed. The challenge will more likely lie in achieving N synchrony between grain crop N demands at seed fill, and supply of N from N mineralization and direct N transfer from legumes.

#### 4.2. N-transfer from legume to crop

There are many potential above- and belowground pathways for N to be transferred from legume to neighboring non-legumes, but the majority of the  $N_2$  fixed by legumes would be expected to be released into soil following the mineralization of N from legume shoot and nodulated root residues following a stress (grazing, mowing, drought) or end of season senescence (Peoples et al., 2015). In native and agricultural ecosystems featuring herbaceous perennial species, it is unclear how much shoot N ultimately makes its way into the soil available N pool. Defoliation of legumes has been found to substantially increase the release of belowground N in the form of root exudates. Ayres et al. (2007) found that direct  $^{15}N$  transfer from roots of white clover (*Trifolium repens*) to roots of perennial ryegrass (*Lolium perenne*) increased five-fold following defoliation while the size of the microbial biomass pool increased by 77%. Following defoliation, the white clover demonstrated strong compensatory growth by increasing shoot and root production by 100% and 34%, respectively. Jørgensen et al. (1999) reported that the rate of N transfer from white clover to perennial ryegrass over the growing season approximately matched the rate of  $N_2$  fixation rate in white clover, indicating





**Fig. 5.** Avenues of N transfer from legume (plant on left) to grain crop. Direct pathways include transfer via mycorrhizal fungi, and movement of inorganic or organic N through bulk soil that were released or recently mineralized from legume tissues. The indirect pathway involves pool substitution in which legume nitrogen is immobilized into microbial biomass or the SOM-N pool, while N is mineralized from the same SOM-N pool and taken up by the grain crop (reproduced with permission from Ayres et al., 2007).

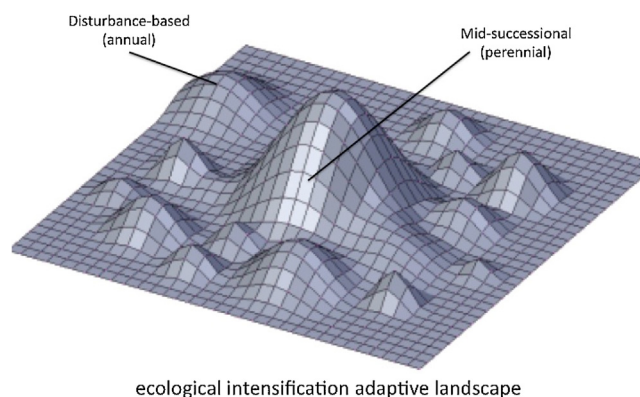
that N availability from clover to grass may be linked to the N status of the clover plant. These findings underscore the importance of both direct transfer of N from legume to grass which is relevant in narrow time frames, and indirect transfer via the increase in microbial biomass which can function as an interim storage of N (Fig. 5; Peoples et al., 2015). These two avenues can also be linked, as legume contributions can result in short-term assimilation into microbial biomass and subsequent mineralization of N from the microbial pool or stabilization of N in SOM with longer turnover times (Liang and Balser, 2010; Murphy et al., 1998).

Grazing livestock at one or more times during the year is a defoliation mechanism that can induce both above- and below-ground N transfer from legume to grass. A  $^{13}\text{C}$  tracer experiment (Hamilton and Frank, 2001) revealed that grazing of plants by livestock stimulated root C exudation, which increased microbial activity in the rhizosphere and the release of plant-available N through decomposition, and subsequent plant N uptake. Ungulates have also been found to stimulate net N mineralization at a landscape scale in natural ecosystems (Frank and Groffman, 1998; McNaughton et al., 1997). In addition, plants can release C in response to nutrient patches (Paterson, 2003; Paterson et al., 2006), suggesting that they may control their C allocation to increase nutrient acquisition. Such evolutionary mechanisms could potentially be selected for while breeding perennial grain species to increase nutrient cycling efficiency.

## 5. Conclusion

Since the beginning of grain agriculture, humanity has scarcely questioned the nature of the food-producing ecosystem that supplies the majority of our calories—indeed we have only recently begun to recognize that our farms are in fact “ecosystems”. A century of agronomic science has helped us to produce more food than ever from these ecosystems, with energy-intensive mechanical and chemical inputs. A century of agronomic and ecological science has also demonstrated how poorly agroecosystems function in many respects relative to the native ecosystems they replaced. Many of the shortcomings appear as a result of low to very low levels of diversity and perenniality. Recent efforts to increase agricultural sustainability through ecological intensification have focused on increasing diversity of crops and crop-supporting species. While essential, the sole focus on diversity is insufficient, as many challenges in agriculture result from the maintenance of agroecosystems in early stages of secondary succession.

Recent research suggests that human activities have increased the amount of reactive N in the terrestrial ecosphere per year by 320% over background rates (Vitousek et al., 2013) pushing us into a high risk overshoot of critical planetary boundaries (Steffen et al., 2015). Erosion and loss of SOM have and continue to degrade soils globally, especially in regions with limited economic resources to



**Fig. 6.** Ecological intensification adaptive landscape in disturbance-based and mid-successional food producing ecosystems. Disturbance based agriculture featuring annual grains is near the top of an adaptive peak in supporting, regulating and provisioning ecosystem services. The taller adaptive peak of mid-successional grain agriculture may hold greater potential for providing these services through ecological intensification, however considerable investment in research will be required to ascend the new summit.

**Table 2**

Anticipated benefits, ecological mechanisms and challenges to the nitrogen economy of mid-successional legume-grain intercrop agroecosystems.

Benefits	Mechanisms	Challenges
Reduced soil erosion Increased soil organic matter (SOM) Greater nitrogen retention	Perennial vegetation cover Reduced SOM mineralization increased root allocation Improved nitrate uptake efficiencies by perennial roots	Avoiding soil loss in establishment year N immobilized in proportion to C Nitrate loss in establishment year
Improved N synchrony between crop N demand and soil N sources	Legume-fixed N introduced gradually into soil via mineralization N stored in the dormant season within perennial crops by translocation Higher functioning soil microbiome (e.g., mineralizing, transferring N)	Achieving adequate soil N supply through microbial mineralization at grain-fill –
N derived from solar rather than fossil energy Reduced N <sub>2</sub> O emissions	Biologically-fixed N from legume Lower soil moisture and nitrate contents	Managing competition between grain and legume species High labile soil C may favor N <sub>2</sub> O emissions

improve management practices (Amundson et al., 2015; Montgomery, 2007). These and other trends have led to calls for much more resource-efficient and resilient agroecosystems (Foley et al., 2011). Yet as we have discussed here, the ability to achieve ecological intensification in chronically disturbed ecosystems is inherently limited. A transition to later successional agroecosystems would constitute a shift in the “adaptive landscape” of agriculture (Fig. 6). Disturbance-based agroecosystems situated near the top of an adaptive peak have limited ability to improve on supporting, regulating, and provisioning ecosystem services. It will require decades of sustained efforts, contending with many false starts and setbacks before arriving at an acceptable contour on the mid-successional peak that features perennial crops. In this paper we focused on potential improvements in N economy with the transition to mid-successional agroecosystems, but other succession related dimensions of ecological intensification are waiting to be explored, including changes in soil structure, the soil microbiome, water balance, and insect, pathogen and weed ecology. Given the multifunctional improvements that may follow from a later successional agroecosystem (Table 2), the journey seems well worth the effort and risk.

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