

Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review

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Abstract Nitrogen is the most limiting nutrient in most agroecosystems and thus critical for sustaining high yields. Conventional agricultural practices use synthetic fertilizers to ensure an adequate supply of nitrogen in soils, but fertilizers come at a significant monetary and environmental cost. A strategy to improve nitrogen supply in cropping systems is the inclusion of nitrogen-fixing legumes, which can provide nitrogen benefits to companion crops through belowground nitrogen transfer. However, a better understanding of the underlying mechanisms and factors that govern nitrogen transfer is important in order to determine potential areas for improving this association. Here, we review the mechanisms of belowground nitrogen transfer in managed herbaceous cropping systems, focusing on forage systems. We classify three major routes of nitrogen transfer from legumes to non-legumes: (1) decomposition of legume root tissues and uptake of mineralized nitrogen by neighboring plants, (2) exudation of soluble nitrogen compounds by legumes and uptake by non-legumes, and (3) transfer of nitrogen mediated by plant-associated mycorrhizae. Literature data shows that rates of nitrogen transfer range from 0 to 73 % from forage legumes to companion grasses in mixed stands, depending on the legume species and cultivar. We list the factors that affect nitrogen transfer including abiotic factors, e.g., water stress, temperature, light,

soil available nitrogen, and application of nitrogen fertilizer, and biotic factors, e.g., root contact, plant density, growth stage, production year, defoliation, and root herbivores. While the rates of nitrogen transfer are often constrained by abiotic conditions, such as temperature and water availability, that are beyond the control of growers, agronomic practices, e.g., planting density and choice of species and cultivar, may help to increase nitrogen transfer. Ultimately, the selection of plant pairs with compatible traits offers the best path forward to improving nitrogen transfer in intercrops.

Keywords Nitrogen transfer · Symbiotic nitrogen fixation · Cropping system · Legume · Non-legume · Root exudate · Decomposition · Mycorrhizae · Mineralization

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1 Introduction

Nitrogen (N) is critical for the growth and development of crop plants, second only to light and water. While most plant species depend on the uptake of soil N to satisfy their needs, certain clades, most notably the legumes, are capable of fixing N via a symbiotic relationship with rhizobia bacteria (Carranca 2013). This fixed N may benefit not only the legumes but also companion/subsequent crops, a phenomenon that has been observed in legume–grass forage mixtures (Thilakarathna et al. 2012b) (Fig. 1), grain legume–cereal intercropping systems (Chapagain and Riseman 2014, 2015) (Fig. 2), and in agroforestry systems (Nygren and Leblanc 2015). Much of the N made available to non-legumes derives

from the breakdown of legume–crop residues; however, results from mixed-cropping systems research suggest that plants may acquire N directly from companion plants in a process termed interplant N transfer (Stern 1993; Johansen and Jensen 1996). N transfer is defined as the movement of N from one living plant (termed an “N donor”) to another (“N receiver”). It is a bi-directional process (Yong et al. 2015), but the net movement of N tends to flow from plants containing relatively high N (i.e., legumes and other N fixers) to those with a greater N demand (non-fixers) (Carlsson and Huss-Danell 2014). N transfer is highly variable and can supply anywhere from 0 to 80 % of a receiver plant’s N demand (Moyer-Henry et al. 2006; He et al. 2009; Chalk et al. 2014).

With increasing pressure for more sustainable farming practices, supplying more N via fixation instead of from synthetic fertilizers has become an attractive option (Paynel et al. 2008; Fustec et al. 2010). However, in order to maximize the benefits of biologically fixed N in agrosystems, a better understanding is required of the factors that affect the efficiency of plant-to-plant N transfer. This review paper primarily addresses three major aspects of belowground nitrogen transfer: (1) major mechanisms of belowground N transfer; (2) different



Fig. 1 Simple and complex legume–grass forage stands containing **a** white clover (*Trifolium repens* L.) with meadow fescue (*Festuca pratensis*) and Kentucky bluegrass (*Poa pratensis* L.); **b** white clover with timothy grass (*Phleum pratense*), Kentucky bluegrass, and reed

canarygrass (*Phalaris arundinacea*); **c** alfalfa (*Medicago sativa* L.) with timothy grass and Kentucky bluegrass; and **d** naturalized pasture sward containing red clover with bluegrass, bentgrass (*Agrostis* spp.), timothy grass, meadow fescue, and creeping red fescue (*Festuca rubra*)

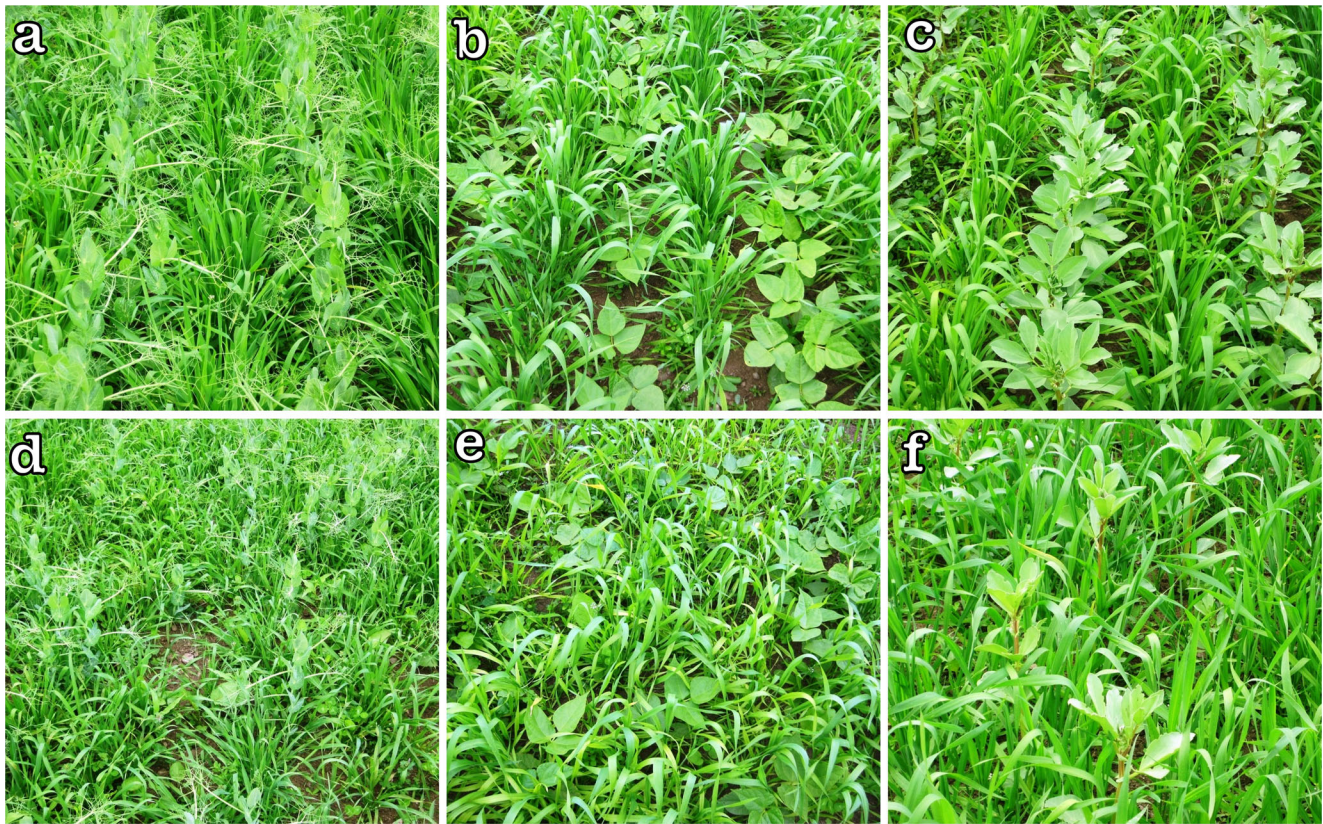


Fig. 2 Cereal-grain legume intercropping systems, **a** barley (*Hordeum vulgare* L.):pea (*Pisum sativum* L.) in rows of 1:1, **b** wheat (*Triticum aestivum* L.):common bean (*Phaseolus vulgaris* L.) in rows of 1:1, **c** wheat:fava bean (*Vicia fava* L.) in rows of 1:1, **d** barley and pea in a

mixed system, **e** wheat and common bean in mixed system, and **f** wheat and fava bean in a mixed system. The significance of N transfer from grain legumes to associated cereal varies according to the legume species as well as the cropping system (mixed/row intercropping)

biotic and abiotic factors that affect N transfer; and (3) genetic variability associated with N transfer between legumes and non-legumes, with a focus on forage-legume-grass systems. As N transfer in agroforestry systems has recently been reviewed (Munroe and Isaac 2014), we focused this review specifically on herbaceous crops within managed agricultural systems. Finally, the review concludes with identifying gaps and providing recommendations for future studies related to N transfer.

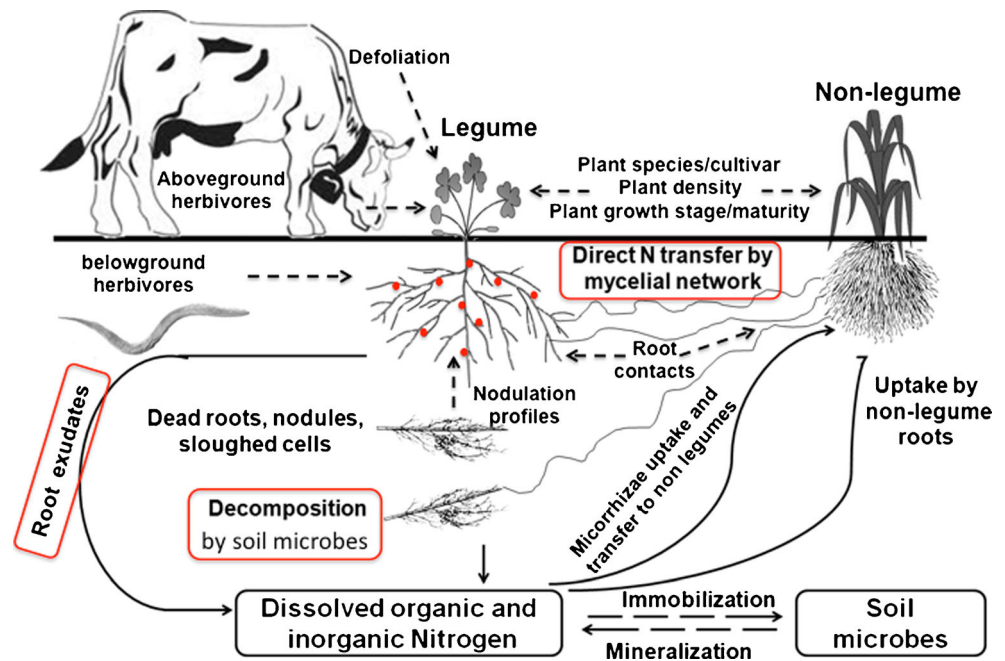
2 Nitrogen transfer mechanisms

The major routes of N transfer can be categorized as above-ground and belowground (Ledgard 1991; Høgh-Jensen and Schjoerring 2000; Rouquette and Smith 2010; Peoples et al. 2015). Aboveground N transfer, not the focus of this review, occurs through shoot litter decomposition and animal excreta through grazing (Ledgard 1991, 2001; Fujita et al. 1992; Warembourg et al. 1997; Dahlin and Stenberg 2010a). Belowground N transfer, the focus of this paper, involves three distinct pathways (Fig. 3):

- (1) *Decomposition*: decomposition of legume root tissues followed by uptake of released N by neighboring plants (Fustec et al. 2010);
- (2) *Root exudation*: the exudation of soluble N compounds by donors and uptake by receivers (Gylfadóttir et al. 2007; Paynel et al. 2008; Wichern et al. 2008);
- (3) *Mycorrhizae*: transfer of N mediated by plant-associated mycorrhizae (He et al. 2003, 2009).

Nitrogen transfer is often described as either “direct” or “indirect,” but not all of these processes fit nicely into a single category. Direct N transfer is the transfer of N from a donor plant to receiver plant without undergoing mineralization, whereas indirect N transfer involves mineralization followed by uptake of N compounds by the receiver. The common mycelial network, which interconnects the root systems of nearby plants (Haystead et al. 1988; Dubach and Russelle 1994; He et al. 2003), is the most direct path for the transfer of N from donor to receiver (Fig. 3). However, mycorrhizae also facilitate indirect N transfer through uptake and transfer of N derived from decomposing organic matter of the donor (e.g., legume roots and nodule debris) (Fig. 3); this can only occur after the root system of the receiver plant is colonized with mycorrhizae (Leigh et al. 2009). The root exudate

Fig. 3 Possible belowground nitrogen transfer mechanisms from legumes to non-legumes (decomposition of roots and nodules, root exudates, and mycorrhizal mediated N transfer) and the wide range of biotic and abiotic factors that affect N transfer (shown with *dashed arrows*)



pathway has been considered both direct (Paynel et al. 2008) and indirect (Jalonen et al. 2009a), depending on whether the N exudate is taken up immediately by neighboring plants (direct N transfer) or initially mineralized by soil microbiota (indirect N transfer). The decomposition pathway can be considered to be the most indirect, as N from root debris must be first decomposed and mineralized before it is accessible for uptake by receiver roots (Fustec et al. 2010).

2.1 Nitrogen transfer through senescence and decomposition of roots and nodules

Nitrogen derived from decomposed roots, nodules, root caps, root border cells, sloughed cells, and the epidermis (water-insoluble materials) significantly contributes to belowground N transfer (Wichern et al. 2008; Fustec et al. 2010; Louarn et al. 2015). When comparing different mechanisms of N transfer, nodule and root decomposition are considered to be more important than root exudates or mycorrhizae-mediated N transfer (Trannin et al. 2000; Sierra et al. 2007) but can vary greatly by legume species (Ta and Faris 1987). It has been estimated that 3 to 102 kg N ha⁻¹ yr⁻¹ of N can be transferred via decomposition of roots and nodules in legumes, equivalent to 2 to 26 % of the biologically fixed N in legumes (Ledgard and Steele 1992). Although most belowground plant tissues contribute to N transfer, the quality of different tissues varies among legume species. Dubach and Russelle (1994) found that decomposing roots release more N than nodules in alfalfa (*Medicago sativa* L.), whereas the opposite trend was observed in birdsfoot trefoil (*Lotus corniculatus* L.). This difference may be due to tolerance by alfalfa nodules to defoliation stress (they remain intact even after harvesting) compared to

birdsfoot trefoil (Vance et al. 1979). Although decomposition of belowground plant tissues contributes significantly to N transfer, it is generally a slow process compared to N transfer through root exudates and mycorrhizae. The former involves decomposition and cycling of complex organic compounds, whereas the latter is the transfer of simple inorganic N (Goodman 1988). As a result, N derived from decomposition of roots and nodules mostly contributes to later stages of plant growth or subsequent production years (see below) (Burity et al. 1989; Jørgensen et al. 1999; Louarn et al. 2015).

2.2 Nitrogen transfer through root exudates

Compounds released by the root system into the surrounding soil are referred to as root exudates (Walker et al. 2003). Root exudates can be either low-molecular-weight compounds (e.g., amino acids, organic acids, sugars, phenolics, and various other secondary metabolites) or high-molecular-weight compounds (e.g., proteins) (Walker et al. 2003; Prithiviraj et al. 2007; Badri and Vivanco 2009). Root exudation of low-molecular-weight compounds can be described as the net balance between the simultaneous discharge of a particular compound from the root (efflux) and its absorption (influx). While efflux is usually described as passive, its release is modulated by membrane-bound ion channels (Badri and Vivanco 2009), just as uptake is controlled by carrier proteins (Segonzac et al. 2007). Studies in hydroponic systems have shown that plants can have a larger degree of control over high-molecular-weight N-compound exudation compared to low-molecular-weight compounds, both in terms of timing and specificity of the compounds that are released (Phillips et al. 2004; Chaparro et al. 2013).

The vast array of functions of root exudates (Dakora and Phillips 2002; Walker et al. 2003; Badri and Vivanco 2009) includes their ability to act as N transfer metabolites (Paynel and Cliquet 2003; Paynel et al. 2008; Jalonen et al. 2009a, b), especially in the short term (Paynel and Cliquet 2003; Gyldfadóttir et al. 2007). At early legume growth stages, the majority of N transfer takes place through root exudates rather than decomposition of roots and nodule debris, as already alluded to (Burity et al. 1989; Lesuffleur et al. 2013). Because plants are able to uptake organic N (amino acids, peptides, and proteins) (Näsholm et al. 2008), root exudates contribute to direct N transfer to non-legumes. On the other hand, exudates can be taken up and mineralized quickly by soil microbes (van Kessel et al. 2009) due to their low C:N ratio (Uselman et al. 2000), which facilitates subsequent (indirect) N transfer (Jalonen et al. 2009a).

Ammonium, followed by amino acids, are the major forms of low-molecular-weight N-containing compounds exuded by most temperate legumes (Paynel et al. 2001, 2008; Paynel and Cliquet 2003; Lesuffleur and Cliquet 2010; Lesuffleur et al. 2013). Among the different amino acids, glycine and serine are the dominant forms in clover root exudates (Lesuffleur et al. 2007; Paynel et al. 2008), but the following amino acids are also observed: glutamate, glutamine, aspartate, asparagine, tyrosine, alanine, valine, arginine, methionine, phenylalanine, leucine, isoleucine, and lysine (Paynel et al. 2008). Amino acid exudation primarily occurs through root nodules and root tips (White et al. 2007; Lesuffleur and Cliquet 2010). The form of N compounds exuded by legumes depends on the type of legume; temperate legumes (e.g., alfalfa) release most of the N as amino-N or $\text{NH}_4\text{-N}$, whereas tropical legumes (e.g., soybean, *Glycine max* L. Merr.) primarily release ureides (Brophy and Heichel 1989; Ofosu-Budu et al. 1990). This difference is because fixed N in temperate legumes is transported through xylem in the form of amides (e.g., asparagine and glutamine), whereas in tropical legumes, fixed N is transported mainly as ureides (Pélissier et al. 2004). Different plant-associated factors affect root N exudation; specifically, N_2 fixation (Paynel et al. 2008), root N concentration (Jalonen et al. 2009a), and total plant N content (Mahieu et al. 2009) were shown to promote N exudates. As the concentration of N compounds exuded by a root system is greater closer to the root system than at a distance (Merbach et al. 1999), close root contact of the legume and non-legume is important for efficient transfer of N-containing exudates.

2.3 Mycorrhiza-mediated nitrogen transfer

Approximately 70–90 % of terrestrial plant species form symbiotic associations with arbuscular mycorrhizal fungi (Pamiske 2008). Most mycorrhizal species exhibit low host specificity (Albrecht et al. 1999), allowing for connections between dissimilar plants to be common. Various studies have demonstrated

that the presence of arbuscular mycorrhizal fungi can increase the transfer of symbiotically fixed N from legumes to non-legumes, including white clover to perennial ryegrass (*Lolium perenne* L.) (Haystead et al. 1988), berseem clover (*Trifolium alexandrinum* L.) to maize (*Zea mays* L.) (Frey and Schuepp 1992), pea (*Pisum sativum* L.) to barley (*Hordeum vulgare* L.) (Johansen and Jensen 1996), soybean to maize (van Kessel et al. 1985; Hamel et al. 1991), and mung bean (*Vigna radiate* L. Wilczek) to rice (*Oryza sativa* L.) (Li et al. 2009).

Mechanistically, arbuscular mycorrhizal fungi can facilitate the transfer of N between plants, either, as already noted, by creating direct mycelial connections between donors and receivers (Haystead et al. 1988; Høgh-Jensen 2006; Meng et al. 2015) or by boosting the capacity of receivers to uptake legume-derived N by increasing the volume of soil they have access to (San-nai and Ming-pu 2000; Høgh-Jensen 2006). Arbuscular mycorrhizal fungi can uptake NH_4^+ , NO_3^- , and organic N (He et al. 2003) and are able to obtain significant amounts of N from decomposing soil organic material (Hamel et al. 1991; Hodge and Fitter 2010), thus making them excellent scavengers of N. Plants colonized with arbuscular mycorrhizal fungi are efficient at intercepting N from the soil system (Cavagnaro et al. 2012; Jannoura et al. 2012) and reducing N losses from the system (van der Heijden and Horton 2009; Asghari and Cavagnaro 2012), suggesting that in legume/non-legume mixed-cropping systems, N released by legumes can be efficiently held by the mycorrhizae and subsequently transferred to neighboring non-legumes.

Direct N transfer through mycorrhizae in legume/non-legume associations can be bidirectional (He et al. 2003, 2009), but the majority of mycorrhizal-mediated N transfer is from N_2 -fixing legumes to non- N_2 -fixing plants (up to 80 % N transfer), whereas less than 10 % of N transfers are from non- N_2 -fixing plants to legumes (He et al. 2009). This difference can be explained by the fact that mycorrhizae-mediated N transfer is driven by source-sink relationships, wherein N transfer takes place from plants with a high N to low N concentration, as noted above (Jalonen et al. 2009b).

3 Factors affecting nitrogen transfer

3.1 Abiotic factors

3.1.1 Water stress

Water availability in the soil can have a profound impact on nitrogen dynamics both within the plant and the soil, making it an important determinant of N transfer. Under conditions of drought, root cell wall permeability often increases, altering the amount and composition of N compounds released into the soil (Brophy and Heichel 1989). In addition, nodule senescence is more common during

periods of water deficit (Gogorcena et al. 1995; Mhadhbi et al. 2011), resulting in greater N available for mineralization, particularly because N accumulation in the nodules occurs during drought stress (Ladrera et al. 2007). Conversely, drought conditions can severely hamper N fixation (Serraj et al. 1999), and dry soils typically do not favor the movement of N, since N mineralization decreases (Fierer and Schimel 2002; Schimel et al. 2007) as does the mass flow of soluble nutrients (Lambers et al. 2008), limiting potential uptake by N receivers. However, reduced uptake of N as a result of drought is usually temporary, as plants can alter their morphology to compensate (He and Dijkstra 2014). Nevertheless, dry conditions can still favor higher rates of N transfer compared to wet conditions (Ledgard 1991). In addition to water scarcity, excess water can also impact N transfer especially through nitrate leaching below the rooting zone (Thilakarathna 2016). Furthermore, under severe flooding conditions, N can be lost as nitrous oxide emissions (Saggar et al. 2004), placing N out of reach of N receiver plants. Potential N losses can be mitigated by N receivers through increasing their density and diversity (Scherer-Lorenzen et al. 2003).

3.1.2 Temperature and light

As with water, light and temperature can have multiple contrasting effects on the supply and demand of N, both at the plant and soil levels. For plants, greater light and temperature generally result in an increased metabolic rate, which in turn drives high rates of N fixation (Fujita et al. 1992) as well as N uptake from N receivers (von Wirén et al. 1997). High light intensity and temperature have also been associated with an increase in N exudation from legumes (Ofosu-Budu et al. 1995a; Schroth et al. 1996), suggesting that both N donors and N receivers are working at a higher efficiency. However, field reports often show that N transfer may be highest during the early and late seasons, when temperatures and light are at their lowest (Gylfadóttir et al. 2007; Rasmussen et al. 2013), a result similar to that observed in controlled conditions (Ta and Faris 1988). During these conditions, soil microflora are also stimulated (Kuzyakov and Xu 2013), making them more efficient competitors for soil N.

Freezing and thawing also can cause senescence and turnover of root and nodule tissues in legumes, which enables high N transfer to non-legumes during the subsequent growing season especially in forage-based cropping systems (Oberson et al. 2013). In addition, prolonged dark conditions affect nodule function and can induce rapid nodule senescence (Hernández-Jiménez et al. 2002; Pérez Guerra et al. 2010). This situation can occur in dense mixed stands (e.g., forage-grass mixed stand), where shade conditions can lead to nodule senescence.

3.1.3 Soil nitrogen availability and application of nitrogen fertilizer

Soil N availability is highly variable, spatially and temporally. Reductions in N transfer under high soil mineral N availability have been reported in legume–non-legume mixtures, including peanut (*Arachis hypogaea* L.)–rice (Chu et al. 2004), white clover–turf grass (Sincik and Acikgoz 2007), white clover–perennial ryegrass (Rasmussen et al. 2013), and soybean–maize relay cropping (Smýkal et al. 2015). These results may be explained by a reduction in legume N₂ fixation under high mineral N levels (Naudin et al. 2010), whereby the rate of N transfer is related to the rate of legume N₂ fixation (Mahieu et al. 2009). Contrary to these findings, some reports have shown that N transfer is greater under conditions of high inorganic N availability (Ofosu-Budu et al. 1995a; Høgh-Jensen and Schjoerring 1997; Elgersma et al. 2000). High N concentrations may increase root growth of the N receiver, providing a larger sink for inorganic and legume-derived N (Ofosu-Budu et al. 1995a; Paynel et al. 2008). In addition to plant growth stimulation, application of inorganic N fertilizer can have priming effects, which leads to rapid mineralization of organic matter and release of further mineral N (Kuzyakov et al. 2000). Van Der Krift et al. (2001) observed that the decomposition rate of roots and rhizodeposits was greater in N rich systems compared to N-deficient systems. Detailed studies across a variety of cropping systems are necessary in order to understand the underlying mechanisms that govern the negative and positive effects of mineral N on N transfer rates.

3.2 Biotic factors

3.2.1 Root contact

The spatial arrangement of root systems in legume/non-legume associations is important for efficient N transfer, as transfer rates tend to be highest when roots are in close proximity, as already noted (Fujita et al. 1990; Ofosu-Budu et al. 1995a; Xiao et al. 2004; Daudin and Sierra 2008; Meng et al. 2015). First, close contact of root systems of legumes and non-legumes reduces the distance that N compounds must travel by mass flow. Second, the concentration of rhizodeposited N is high close to the root rhizosphere and decreases rapidly away from the rhizosphere (Merbach et al. 1999; Schenck zu Schweinsberg-Mickan et al. 2010; Rasmussen et al. 2013). Furthermore, the rhizodeposited N concentration from legumes decreases with soil depth, while the majority of the rhizodeposited N (95 %) is located in the topsoil layer (0–15 cm) (Høgh-Jensen and Schjoerring 2001; Laberge et al. 2011), where the root density is higher compared to deep soil layers.

A legume's root architecture also influences the efficiency by which its roots contact neighboring non-legumes and permit subsequent N transfer. For example, alfalfa has a high N₂ fixation capability compared to other forage legumes, but the associated N transfer is lower, which may be related to its relatively low number of lateral roots (Pirhofer-Walzl et al. 2012). Alfalfa has a deep taproot and fewer secondary roots, which limits close root contact with neighboring plants (Chmelíková and Hejcman 2012), especially when compared to white clover which has a highly branched root system.

3.2.2 Legume–non-legume plant density

The composition of plant species in a mixed stand affects the efficiency of N transfer from legumes to non-legumes (Høgh-Jensen and Schjoerring 1997; Chapagain and Riseman 2014, 2015; Suter et al. 2015). Generally, N transfer is higher in a legume–non-legume mixed stand when the legume fraction is greater than the non-legume fraction, as observed in different legume–grass mixtures including alfalfa–birdsfoot trefoil–reed canarygrass (*Phalaris arundinacea* L.) (Brophy et al. 1987), white clover–red clover–birdsfoot trefoil–tall fescue (*Festuca arundinacea*) (Mallarino et al. 1990), and seven different tropical forage legumes with *Brachiaria brizantha* (Viera-Vargas et al. 1995). A higher legume ratio can be altered through increased seeding with legumes (Høgh-Jensen and Schjoerring 1997) or narrower row spacing (Haby et al. 2006). Chapagain and Riseman (2014, 2015) and Li et al. (2015) have shown that total soil N and available soil N were higher when a legume and companion grass were planted in 1:1 rows, whereas these values decreased as the proportion of non-legumes increased. Greater N transfer was found in a 1:1 ratio (11 % N transfer) of barley:pea compared to a 2:1 ratio (4 % N transfer) (Chapagain and Riseman 2014). A higher legume:non-legume plant density favors intermingling of plant roots, mycorrhizal connections, and reduced distance for N movement compared to lower densities, as discussed in the previous section. A higher density of legume roots also results in increased N rhizodeposition (Mahieu et al. 2009). Therefore, the ratio of legume to grass plants can be optimized to achieve maximum N transfer in mixed stands.

The non-legume component in a mixed stand also plays a key role, as it rapidly depletes the available N in soil, resulting in higher N₂ fixation in companion legumes (Viera-Vargas et al. 1995). These stimulatory interactions between the two functional groups (legumes and non-legumes) will enhance the total N yield in the mixed stand (Nyfeler et al. 2011). A potential challenge to keeping the plant density at high levels in an intercrop is exclusion by allelopathy (Ehrmann and Ritz 2013), a trait which is present in potential legume N donors such as red clover (*Trifolium pratense*) (Liu et al. 2013) and alfalfa (Hegde and Miller 1990), as well as N receivers such as red fescue (*Festuca rubra*) (Bertin et al. 2003) and perennial

ryegrass (*L. perenne*) (Chung and Miller 1995). Breeding can reduce the allelopathic potential of crop plants and their resistance to the allelochemicals of nearby plants (Miller 1996), which may be an important factor in improving N transfer between legumes and grasses.

3.2.3 Plant growth stages and production years

The growth stage/pattern of plants and the production year of a field (especially stands with perennials) can affect N transfer rates between legumes and non-legumes (Fujita et al. 1990; Heichel and Henjum 1991; Jensen 1996; Høgh-Jensen and Schjoerring 2000; Frankow-Lindberg and Dahlin 2013). A soil incubation study with pea roots demonstrated that rhizodeposited N at early growth stages (7 weeks) was more labile (mineralizable) compared to the rhizodeposits at maturity (14 weeks) (Jensen 1996). By contrast, Zang et al. (2015) found that N transfer slightly increased from the beginning of pod setting (7.6 %) to maturity (9.7 %) in a mungbean (*V. radiate* L.)–oat intercropping system. The growth pattern of a grass in a legume–grass intercrop can also affect N transfer rates, wherein a grass species with early maturity and rapid growth will more effectively compete for legume-released N (Ta and Faris 1987).

With respect to the production year of a field, N transfer from forage legumes has been shown to be generally higher at latter production years compared to the establishment year or post-establishment year; this has been observed from white clover to ryegrass and from alfalfa to tall fescue (*F. arundinacea*) (Mallarino et al. 1990; Høgh-Jensen and Schjoerring 1997; Jørgensen et al. 1999; Elgersma et al. 2000; Frankow-Lindberg and Dahlin 2013). For example, Jørgensen et al. (1999) found that the apparent transfer of clover N to grass was negligible in the seeding year but increased to 19 and 28 kg N ha⁻¹ in the first and second production years, respectively. In a barley–pea intercropping system, N transfer to barley increased during the second year (16 % transfer rate) compared to the first year (6 % rate) on the same land (Chapagain and Riseman 2014). The authors also showed higher N transfer during the second production year, compared to the first year, in a wheat (*Triticum aestivum* L.)–kidney bean (*Phaseolus vulgaris* L.) intercrop (6 vs. 3 %, respectively) and a wheat–fava bean (*Vicia fava* L.) intercrop (13 vs. 11 %, respectively), on the same land (Chapagain and Riseman 2015). This phenomenon has been explained by increasing decomposition and mineralization of legume root and nodule tissues coupled with grass uptake of mineralized N (Heichel and Henjum 1991).

Nitrogen transfer has also been shown to increase during an individual growing season as the season advances (Dahlin and Stenberg 2010a; Thilakarathna et al. 2012b; Rasmussen et al. 2013). For example, the proportion of N in perennial ryegrass derived from N transfer from red clover increased from the

first harvest (10.1 % N transfer rate) to the third harvest (22.7 %) of clover (Dahlin and Stenberg 2010a); in Kentucky bluegrass (*Poa pratensis* L.), the proportion increased from 7 % in the first harvest of clover to 26 % in the third harvest (Thilakarathna et al. 2012b). Increased N release from the roots of older legume root systems can be attributed to increased root mass, surface area, and exudates, as well as root and nodule senescence and decay (Fustec et al. 2010).

3.2.4 Defoliation stress

Defoliation is the removal of aboveground plant materials, associated with grazing, mowing, frost, insect damage, or herbicide application. Defoliation of legume plants enhances N transfer to neighboring non-legumes (Ayres et al. 2007; Tarui et al. 2013). Furthermore, the defoliation frequency of the legume has a positive relationship with N transfer rates to companion grasses (Høgh-Jensen and Schjoerring 1994), although other studies contradict these results (Dahlin and Martensson 2008; Dahlin and Stenberg 2010b; Frankow-Lindberg and Dahlin 2013). For example, it was found that defoliation of hairy vetch (*Vicia villosa* Roth.) increased N transfer to oat by 26 % compared to the absence of defoliation in a mixed crop (Tarui et al. 2013). Another study which simulated aboveground herbivory showed that shoot removal increased N transfer from white clover to perennial ryegrass (Ayres et al. 2007). As a direct effect, defoliation can increase exudation of different nitrogen compounds from the root system (Ofosu-budu et al. 1995b, c). However, Saj et al. (2008) suggest that defoliation primarily affects direct N transfer from

the legume to grass in a legume–grass mixture instead of altering available soil organic N. Defoliation adds not only N but also carbon into the rhizosphere, which increases microbially mediated N mineralization (Ayres et al. 2004, 2007). Ofosu-budu et al. (1995c) have shown that N release from the root system is closely related to the ATP concentration of the roots, such that a reduction in the root ATP concentration positively correlates with exudation of N compounds. Defoliation removes the primary sink for N, which can lead to the release of N compounds from the legume root system into the rhizosphere via exudation (Hamilton et al. 2008; Carrillo et al. 2011). Recently accumulated N in legume nodules can be released by passive leakage after shoot harvest (Brophy and Heichel 1989). Furthermore, defoliation can induce root and nodule senescence, which in turn lead to their decomposition and mineralization (Chesney and Nygren 2002). Root-derived N can be available in the soil even 8 months after defoliation (Carrillo et al. 2011) as a source of nitrogen for subsequent crops.

3.2.5 Root herbivory

Root herbivory (i.e., the parasitism of roots by nematodes and other soil fauna) can affect N transfer rates, most typically by increasing the rate of root exudation (Bardgett et al. 1999). Larval damage facilitates N release from damaged nodules while increasing nodulation (Ryalls et al. 2013). The amount of N transferred from legumes to neighboring non-legumes varies with the density of root parasites that infect legume roots (Bardgett et al. 1999; Dromph et al. 2006). For example,

Table 1 Effect of legume donor species on nitrogen transfer

Legume species	Grass species	N in grass transferred from legume (% N)	Amount of N transferred	Remarks	Reference(s)
<i>Trifolium repens</i> L.	<i>Festuca arundinaceae</i> L.	Y1 29 % Y2 60 %	Y1 24.7 kg N ha ⁻¹ Y2 50.9 kg N ha ⁻¹	Two-year field study Method ¹⁵ N dilution technique	Mallarino et al. (1990)
<i>Trifolium pratense</i> L.		Y1 27 % Y2 54 %	Y1 21.7 kg N ha ⁻¹ Y2 31.8 kg N ha ⁻¹		
<i>Lotus corniculatus</i> L.		Y1 29 % Y2 55 %	Y1 21.6 kg N ha ⁻¹ Y2 47.0 kg N ha ⁻¹		
		(mean N transfer)	(seasonal transfer)		
<i>Medicago sativa</i> L.	<i>Phalaris arundinacea</i> L.	Y2 29 %	–	Four-year field study	Heichel and
<i>Lotus corniculatus</i> L.		Y2 47 %	–	Method ¹⁵ N dilution technique	Henjum
<i>Trifolium repens</i> L.		–	–	Highest N transfer found	(1991)
<i>Trifolium pratense</i> L.		–	–	during the second year	
<i>Trifolium repens</i> L.	Grass and herb multi-	–	4.8 g N m ⁻²	Field study	Pirhofer-Walzl
<i>Trifolium pratense</i> L.	species	–	2.2 g N m ⁻²	Method ¹⁵ N leaf labeling technique	et al. (2012)
<i>Medicago sativa</i> L.		–	1.1 g N m ⁻²		
<i>Trifolium repens</i> L.	<i>Lolium perene</i> L.	–	147 kg N ha ⁻¹	Three-year field study	Louarn et al.
<i>Medicago sativa</i> L.	<i>Festuca arundinaceae</i> Shreb.	–	59 kg N ha ⁻¹ (cumulated N transferred across 3 years)	Method ¹⁵ N dilution technique	(2015)

C cut/harvest number, Y production year

Table 2 Effect of legume cultivar type on nitrogen transfer

Legume species	Legume cultivar	Grass species	N in grass transferred from legume (% N)	Amount of N transferred	Remarks	Reference(s)
<i>Trifolium repens</i> L.	Grasslands-Huia Grasslands-Pitau Grasslands-Kopu Aran	<i>Lolium perenne</i> L.	No cultivar differences	Up to 70 kg N ha ⁻¹	One-year field study with dairy cow grazing. Method ¹⁵ N dilution technique	Ledgard (1991)
<i>Trifolium pratense</i> L.	APR-E701 Arlington Mammoth Redland II	<i>Dactylis glomerata</i> L.	Y1 44.3 % Y2 70.5 % Y1 45.4 % Y2 68.6 % Y1 43.7 % Y2 65.9 % Y1 48.9 % Y2 69.6 % (seasonal mean)	Y1 15.7 Y2 59.2 Y1 16.3 Y2 57.0 Y1 17.6 Y2 53.0 Y1 17.6 Y2 62.0 (kg N ha ⁻¹)	Two-year field study with three harvests in each year Method ¹⁵ N dilution technique	Farnham and George (1993)
<i>Trifolium repens</i> L.	Aran	<i>Lolium perenne</i> L.	–	Y1 26.0 Y2 78.7 Y3 49.2 Y4 32.3 Y1 40.3 Y2 83.8 Y3 74.9 Y4 52.2 Y1 44.3 Y2 75.8 Y3 74.1 Y4 57.9 (kg N ha ⁻¹)	Four-year field experiment with three white clover cultivars with different leaf sizes. Method ¹⁵ N dilution technique	Laidlaw et al. (1996)
<i>Trifolium repens</i> L.	Alice Gwenda	<i>Lolium perenne</i> L.	–	Y1 64 Y2 87 Y1 61 Y2 115 (kg N ha ⁻¹)	Two-year field study with zero-N and two-N concentrations (150, 180 kg N ha ⁻¹) Method Nitrogen difference method	Elgersma et al. (2000)
<i>Trifolium pratense</i> L.	AC Christie Tempus	<i>Poa pratensis</i> L.	C1 5.5 % C2 10.5 % C3 23.6 % C1 7.6 % C2 11.6 % C3 28 %	C1 186 C2 237 C3 400 C1 225 C2 283 C3 440 (mg N plant ⁻¹)	One-year field study with three cuts, using two red clover cultivars Method ¹⁵ N dilution technique	Thilakarathna et al. (2012b)
<i>Trifolium pratense</i> L.	AC Christie	<i>Poa pratensis</i> L.	–	Y1 29.7 Y2 60.0	Two-year field study with three cuts, using six red clover cultivars	Thilakarathna et al. (2016)

Table 2 (continued)

Legume species	Legume cultivar	Grass species	N in grass transferred from legume (% N)	Amount of N transferred	Remarks	Reference(s)
	Tapani			Y1 0 Y2 36.4	Method ^{15}N dilution technique Genotypic variability among different red clover cultivars was found for N transfer. Generally N transfer increased during subsequent production years	
	CRS 15			Y1 47.2 Y2 18.9		
	Tempus			Y1 45.0 Y2 90.9		
	CRS 39			Y1 12.0		
	CRS 18			Y2 73.5		
				Y1 4.0		
				Y2 0		
				(mg N plant $^{-1}$)		

C cut/harvest number, Y production year

white clover roots infested at low densities increased the leakage of N into the rhizosphere (Bardgett et al. 1999). A significant (37 %) increase in N content of perennial ryegrass has been reported as a result of root herbivore-induced N transfer from white clover (Hatch and Murray 1994). In contrast to these observations, Ayres et al. (2007) observed a reduction in N transfer between white clover and perennial ryegrass as a result of herbivore nematode (*Heterodera trifolii*) damage to clover roots.

4 Plant genetic variability for nitrogen transfer

4.1 Forage legume species

Species-specific differences in N transfer rates among pasture legumes have been widely reported (Table 1). For example, Pirhofer-Walzl et al. (2012) found that white clover transferred more N (4.8 g m^{-2}) to neighboring plants compared to red clover (2.2 g m^{-2}) or alfalfa (1.1 g m^{-2}). The same trend was found by Frankow-Lindberg and Dahlin (2013) and Louarn et al. (2015) who observed that red clover and white clover transferred more N than alfalfa. A higher rate of N transfer was also reported from white clover to tall fescue compared to birdsfoot trefoil and red clover (Mallarino et al. 1990). Heichel and Henjum (1991) reported a higher percentage of grass N from birdsfoot trefoil (47 %) compared to alfalfa (29 %) (during year 2).

Differences between legumes in their growth habits, root traits, nodulation profiles, root exudation profiles, decomposition rates, and mycorrhizal associations may explain the different rates of N transfer observed among legume species. Based on the above studies, white clover appears to transfer more N compared to other pasture legumes, with alfalfa showing the lowest N transfer rate. White clover multiplies from its stolons, bypassing the need to store N within the root system, thus making available more N from its roots for N transfer. Generally, harvesting accelerates the death and rapid turnover of white clover's stolons (Sturite et al. 2007), which in turn contributes to N transfer. Furthermore, the root system of white clover has a high density of fine roots, a low C:N ratio, and low lignin content; all of which accelerate root turnover (Louarn et al. 2015). By contrast, alfalfa invests more resources in maintaining its perennial nature (Louarn et al. 2015), which includes storing N in its taproot system to ensure regrowth after defoliation (Pirhofer-Walzl et al. 2012). Furthermore, alfalfa develops poor or small secondary root systems (Louarn et al. 2015), which further limits close root contact with non-legumes. In addition, alfalfa has a thick root system, high root C:N ratio (>20), and high lignin content in its fine roots and nodules compared to other forage legumes, and these factors also limit root turnover rates (Louarn et al. 2015). It has been demonstrated that tap-rooted grain legumes

tend to have a higher root N concentration than typical pasture legumes, which may lead to a higher rate of N transfer in the long term (Carranca et al. 2015).

4.2 Forage legume cultivars

Nitrogen transfer from legumes to non-legumes is also affected by legume cultivar identity (Table 2). Based on a 4-year field experiment, Laidlaw et al. (1996) observed genotypic variability in rates of N transfer to perennial ryegrass from three different white clover cultivars. Specifically, the white clover cultivar (Aran) with large leaves transferred less N (15 %) than the cultivar with small leaves (Kent Wild, 34 %) (Table 2). Similarly, using red clover, Elgersma et al. (2000) found that a small-leaf cultivar transferred more N (115 kg N ha⁻¹) than a large-leaf cultivar (87 kg N ha⁻¹) during the second field year (Table 2). Genotypic variability was also reported among six different red clover cultivars for N transfer to bluegrass (Thilakarathna et al. 2016). The suggested mechanisms for this genetic variability have been proposed to include slow decay of roots and stolons in the large-leaf

cultivars (Laidlaw et al. 1996); effective competition from the large-leaf cultivars with grasses for available N (Laidlaw et al. 1996); differences in root and nodulation profiles (Thilakarathna et al. 2012a); and a higher herbage-to-root ratio of large-leaf cultivars compared to small-leaf cultivars (Seker et al. 2003), causing N to be diverted from roots (the immediate source of available N for transfer) to shoots. It is important to note that a few studies have shown a lack of variation between cultivars for belowground N transfer, including the following systems: white clover-perennial ryegrass (Ledgard 1991), red clover-orchard grass (*Dactylis glomerata* L.) (Farnham and George 1993), and red clover-bluegrass (Thilakarathna et al. 2012b) (Table 2).

4.3 Non-legume species

The non-legume components of mixed stands also affect N transfer rates (Table 3). Marty et al. (2009) have shown genotypic differences between two grass species (*Festuca eskia* and *Nardus stricta*) for receiving N from alpine clover (*Trifolium alpinum*) in a mixed stand; in this study, 15 % of

Table 3 Effect of grass receiver species on nitrogen transfer

Legume species	Grass species	N in grass transferred from legume (% N)	Amount of N transferred	Remarks	Reference(s)
<i>Medicago sativa</i> L.	<i>Phleum pratense</i> L. (cv. Climax)	Y1C2 31 %	Y1 3.46	Three-year field study Method ¹⁵ N dilution technique No cultivar differences during cut 1 at first 2 years and three cuts at third year	Burity et al. (1989)
		Y2C2 44 %	Y2 13.73		
	Y3 14.90				
	<i>Phleum pratense</i> L. (cv. Salvo)	Y1C2 33 %	Y1 3.51		
Y2C2 55 %		Y2 19.64			
	<i>Bromus inermis</i>	Y1C2 47 %	Y1 6.66		
		Y2C2 57 %	Y2 27.12		
		(only significant results are listed)	Y3 25.49 (kg N ha ⁻¹) (seasonal transfer)		
<i>Trifolium repens</i> L.	<i>Lolium perenne</i> L.	73 %	4.1	Three-year field study with four N concentrations Method N difference method	Sincik and Acikgoz (2007)
	<i>Poa pratensis</i> L.	50 %	1.6		
	<i>Agrostis stolonifera</i> L.	49 %	2.3 (g N m ⁻² yr ⁻¹) (3-year average under 0 N)		
<i>Trifolium alpinum</i> L. (alpine clover)	<i>Festuca eskia</i>	15 %	–	Pot experiment using the plant stands (monoculture and mixed culture) grown in the field Method ¹⁵ N leaf labeling technique	Marty et al. (2009)
	<i>Nardus stricta</i>	1 %	–		
<i>Trifolium pratense</i> L.	<i>Phleum pratense</i> <i>Lolium perenne</i>	Y2C1 15 %	Y2C1 1.37	Two-year field trial with two grass species. Nitrogen transfer from red clover to two grass species was different but followed the same trend across the different cuts	Frankow-Lindberg and Dahlin (2013)
		Y2C2 6 %	Y2C2 0.47		
		Y2C3 28 %	Y2C3 0.58		
		Y2C4 7 %	Y2C4 0.15		
		Y2C1 19 %	Y2C1 3.8		
		Y2C2 7 %	Y2C2 0.73		
		Y2C3 23 %	Y2C3 1.58		
Y2C4 16 %	Y2C4 1.37 (kg N ha ⁻¹)				

C cut/harvest number, Y production year

N was transferred to *F. eschia* while only 1 % was transferred to *N. stricta* (Table 3). Similarly, Sincik and Acikgoz (2007) found differences in N transferred from white clover to perennial ryegrass (73 % rate of N transfer), Kentucky bluegrass (50 %), and creeping bentgrass (*Agrostis stolonifera* L.) (48 %). It was observed that brome grass (*Bromus* spp.) was more efficient than timothy grass (*Phleum pratense* L.) at uptaking N from alfalfa under field conditions (Burity et al. 1989) (Table 3). Perennial ryegrass was similarly more effective than timothy grass at deriving N from red clover (Frankow-Lindberg and Dahlin 2013) (Table 3). In general, grasses with fibrous root systems are more efficient at capturing available N compared to dicotyledonous plants with taproot systems and hence better competitors for acquiring N from legumes (Pirhofer-Walzl et al. 2012; Frankow-Lindberg and Dahlin 2013). In addition to root architecture, other traits that may be responsible for the variation observed between receiver plants for N acquisition from legumes include differences in plant growth rate and mycorrhizal associations. In general, more study is needed in this area, especially to understand variation (or lack of variation) in N uptake between cultivars.

5 Conclusion

Nitrogen transfer from legumes to non-legumes plays a significant role in legume-based agricultural cropping systems. N transfer has the potential to reduce the use of synthetic inorganic N fertilizers and thus support sustainable agriculture. The three major routes of belowground N transfer are (1) uptake of mineralized N from legume root tissues by neighboring plants, (2) exudation of soluble N compounds by legumes then uptake by non-legumes, and (3) plant-associated mycorrhizae-mediated N transfer. Long-term N transfer occurs primarily through decomposition of roots and nodules, while short-term N transfer occurs primarily through N-containing root exudates and mycorrhizae. Nitrogen transfer is influenced by a wide range of biotic factors (root contact, plant density, growth stage, production year, defoliation, and root herbivores) and abiotic factors (water stress, temperature, light, and soil available N).

While much of the research into N transfer has thus far focused on agronomic practices and the choice of species, genetic selection of both N donors and receivers in tandem provides the most promising route for improved N transfer. Because of the complex nature of N transfer and the multiple routes it can take, focusing on one side of the donor-receiver relationship will not be sufficient to reach its full potential. Legume crops must be able to provide a source of N that will be available for non-legumes when they require it, which is the product of multiple traits (e.g., nodulation/N fixation, fine root production/turnover, exudation rates). Non-legumes must

have the capacity to efficiently uptake legume-derived N, also the result of many traits (e.g., N-uptake efficiency, root length/surface area, mycorrhizal competence). Both the legume and non-legume should have growth habits, both aboveground and belowground, that allow for close proximity to enable N transfer and minimal competition. In addition, it must be recognized that soil conditions, both biological and mineral, play critical roles in influencing the rate of N transfer.

Ultimately, in a given agro-ecosystem, it will be critical to determine (a) the major route(s) of N transfer from donors to receivers and (b) the limiting step in that transfer. This calls for a more holistic approach to track the fate of fixed N from the nodule onward and to identify how donors and receivers can be improved to facilitate better transfer of this valuable resource.

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