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Commentary

The nitrogen transfer between plants: An important but difficult flux to quantify

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The exchange of nutrients, nitrogen in particular, between closely associated plants has attracted considerable interest due to its importance in agroecosystems under low external nutrient-input management. The intuitive observation of farmers that grasses benefit from near associations with clovers has not been easy to quantify, mainly because (i) the net effect is measured against large background fluxes, and (ii) excluding one species from one agroecosystem change the system fundamentally. The study of Moyer-Henry et al. (pp. 7–20 in this issue) approaches this problem elegantly by choosing a soil with a relatively low background mineralisation of nitrogen, while maintaining the same species in the system, although in one case as a non-nodulating variety. Their study confirms that substantial inter-plant Nitrogen transfer occurs. The study does, however, also raise the old question of direct and/or indirect transfer pathways (Virtanen et al., 1937; Wilson and Wyss, 1937). The ^{15}N-natural-abundance technique may not be accurate enough to give reliable data on N fluxes, because it is even more sensitive than ^{15}N-isotope dilution techniques to inhomogeneous distributions of tracers and roots (Luxhøj et al., 2003). The use of dual or triple tracers have been used in other studies (Kuzyakov, 2001) but it would be appropriate to apply direct plant labelling techniques using dual or triple tracers to obtain accurate information on this important process under field or semi-field conditions.

Nitrogen-transfer studies in light of high fluxes of nitrogen in the growth media

The role of nitrogen transfer from the legume to the non-legume in the overall grassland Nitrogen cycling was initially estimated by difference methods (Bland, 1967; Simpson, 1976). Techniques based on dilution of soil ^{15}N were developed to get estimates with higher precision (Vallis et al., 1967) and substantial process-oriented information has been obtained by these techniques under field conditions (see references in Høgh-Jensen and Schjoerring, 2000). However, both the difference methods and the ^{15}N-isotope-dilution techniques are sensitive to changes in soil biology and chemistry and influenced by competitive traits like root distribution in the soil profile. As transfer classically has been measured by comparing one system with the legume and one system without (Ta and Faris, 1987; Vallis et al., 1967), both methods are subject to errors because of a possible stimulation of Nitrogen uptake by the non-legume in the mixture, a so-called N sparring effect (Chalk, 1998). Based on a direct ^{15}N-leaf-labelling technique of individual plants, Høgh-Jensen and Schjoerring (2000) found that the ^{15}N-dilution technique does not give reliable estimates of Nitrogen transfer on a short-term basis, and may substantially underestimate net N transfer.

In addition to these concerns, the effect must be viewed as the net effect of potentially much larger fluxes through several Nitrogen pools in the growth media (Ledgard et al., 1998). Using ^{15}N-pool dilution techniques, the inorganic Nitrogen pools have been shown to be extremely dynamic. Below a grassland sod, there was actually no build-up of NO_3^- in the soil as it is absorbed by plants and microorganisms as rapidly as it is pro-

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duced (Jackson et al., 1989). This study found the NH_4^+ pool to be fairly constant and relatively larger than the nitrate pool, but still with a turnover time of approximately 24 h. Following this picture, it is very likely that transfer goes in the direction of a gradient and that this gradient changes dynamically with time. Accordingly, Høgh-Jensen and Schjoerring (2000) concluded that Nitrogen transfer is a bi-directional process affected by both facilitation and competition processes in the mixtures. Earlier comparative studies did not clearly reach this conclusion as they labelled the whole plant community (e.g., Ta et al., 1989).

The interspecific competition in mixtures is generally always strong, so the position of the individual plant and its capacity to take up and absorb a flux of inorganic nitrogen may determine the direction of the transfer. Høgh-Jensen et al. (1997) investigated both the affinity and the maximum influx rate of nitrate and ammonium. White clover and perennial ryegrass have similar affinities for nitrate, but white clover has a much lower affinity for ammonium than ryegrass. White clover has a lower maximum influx rate than ryegrass for both nitrate and ammonium. As most studies have demonstrated that microbes are generally much better competitors than plants for inorganic Nitrogen added from external sources (Jackson et al., 1989; Zak et al., 1990) these kinetic characteristics may not be determinant before the microbial biomass is ‘saturated’ to some extent. The negative values of transfer calculated on the basis of the ^{15}N-isotope-dilution technique (Høgh-Jensen and Schjoerring, 1994, 1997) may indicate that such an ‘unsaturated microbial biomass’ holds for most of the first growing season. It must currently be assumed that the ‘degree of saturation’ depends on the carbon input into the system. So the question is to what degree these findings can be extrapolated to annual crop mixtures? The answer may be sought in combination with the complexity of carbon translocation from the atmosphere into root growth, root respiration, and root excretions (Gorissen, 1996; Kuzyakov, 2001).

The potential role of dissolved organic nitrogen (DON) in transfer processes

So far, nitrate has been considered the mobile nitrogen component in agricultural soils. However, a recent review by Jones et al. (2005) concludes that dissolved organic nitrogen (DON) constitutes a major soluble N pool in most ecosystem soils. This conclusion is partly based on the amount of DON washed out of the root zone of grassland sods where the DON can be of similar magnitude as the inorganic nitrogen pools. Very little is known about leaching of DON from cropped systems, but recent findings indicate that the DON-component is severely underestimated (McTiernan et al., 2001; Siemens and Kaupenjohann, 2002; Siemens et al., 2003).

As DON is present in leachates, plants must have the potential to access some of these pools in the soil (Jones et al. 2005) but direct proof that plant uptake of DON constitutes a major nitrogen source for crops in agricultural settings is still lacking. However, its potential flux into plants has been clearly demonstrated (Näsholm et al., 2000) and recently Thornton and Robinson (2005) showed the importance of considering multiple Nitrogen forms in N acquisition studies. In addition, Persson and Näsholm (2002) found a lack of down-regulation of the amino acid uptake by improved plant Nitrogen status of conifers. We now require experiments that solve the problems associated with current experimental techniques, which accurately reflect soil conditions, and which incorporate measurement of both inward and outward plant organic and inorganic Nitrogen fluxes (Jones et al., 2005). The role for DON is not yet clarified and creative studies may add new information regarding the transfer processes between plants. Näsholm et al. (2000) attempt to use sterile environments to study organic nitrogen uptake by plants. If similar environments could be applied in ^{15}N and ^{14}C tracer studies it would enable the distinction of carbohydrate transfer and amino acid transfer.

The natural ^{15}N-abundance technique

The ^{15}N isotope dilution technique is strongly influenced by small differences in the spatial and temporal distribution of soil ^{15}N which can lead to large errors in the estimates (Chalk and Ladha, 1999; Domenach and Corman, 1984; Shearer and Kohl, 1986) and this warning is particularly relevant when working with natural abundances of the ^{15}N tracer. Under conditions where a natural ^{15}N enrichment of more than 3–5% relative to atmospheric N_2 is obtainable in the newly mineralised inorganic nitrogen, the natural ^{15}N abundance may be a very useful method to estimate the biological N_2 fixation and the transfer of inorganic Nitrogen (Høgh-Jensen and Schjoerring, 1994). This conclusion is, however, drawn on the basis of semi-perennial crops, i.e. grass-clover. Evaluated on the basis of the first cropping season of this study, it must be concluded that the values of transfer calculated on the basis on the ^{15}N-dilution technique vs. the natural ^{15}N-abundance do not agree when comparing the ^{15}N enrichment of the non-legume in mixtures with legumes and in pure stand. Moyer-Henry et al. (pp. 7–20 in this issue) elegantly overcame this problem by using non-nodulating varieties so that they compared mixtures with mixtures. However, this is a unique situation as non-nodulating varieties are not available for most legume crops, and it is an open question to what degree the nitrogen conditions in the soils of the two systems can be compared.

Direct and indirect transfer pathways

Generally there are three pathways for nitrogen transfer in plant mixtures. First, there is the direct transfer through arbuscular-mycorrhizal fungi linking two plants, like legumes and grasses (Bethlenfalvay et al., 1991; Frey and Schiiepp, 1992; Johansen and Jensen, 1996). Secondly, the transfer may take place as nitrogen-containing leaf leachates or rood exudates from one plant reach the second plant's roots in the growth media (Paynel et al., 2001) This may be mediated through mycorrhizal hyphal uptake and translocation (Kapulnik and Douds, 2000; Smith and Read, 1997). Third, the transfer can be indirect as it enters the turnover processes in soil, and possibly also the degradation processes of dead nodules and dead above- or belowground plant tissue (Høgh-Jensen and Schjoerring, 2001; Ledgard and Steele, 1992; Tobita et al., 1994).

Mycorrhizal fungi represent an interface between plants and soils, as they mediate uptake and translocation of nutrients from soil to mycorrhizal roots (Smith and Read, 1997). Plants that have mycorrhizal associations are at a distinctive advantage compared with roots from non-mycorrhizal plants, particularly in Nitrogen-limiting ecosystems where most soil Nitrogen cycling will largely occur due to the turnover of above- and below-ground plant material and decaying soil microorganisms and animals. The degree of this advantage is clearly demonstrated in the study of Moyer-Henry et al. (pp. xx-xx in this issue) and Mårtensen et al. (1998), although other studies question the actual role of mycorrhizal hyphae and the importance in an agroecological context (He et al., 2003; Smith and Read, 1997). Rovira (1959) demonstrated many years ago the significance of root exudates and thus the potential importance in nitrogen transfer. Later, Wacquant et al (1989) confirmed that mixtures periodically excrete nitrogen-containing compounds. Recently, Paynel et al. (2001) found leachates from both clover and grasses to contain significant amounts of ammonium and minor amounts of amino acids, in agreement with other studies. So, this pathway is potentially important and in closely intermingled species, the ammonium must be viewed as prone to competition.

Several studies have demonstrated the death rate of nodules, roots, root cortex, and shoots (Dubach and Russelle, 1994; Goins and Russelle, 1996). Its importance is undisputed but reliable quantitative data are sparse. Therefore, creative studies under field or semi-field conditions like those of Moyer-Henry et al. (pp. xx-xx, this issue) are much needed. This study suggests that arbuscular mycorrhizal fungi play a very important role in the exchange of nutrients, either during direct linking the plants or through enhancing the inorganic nitrogen and organic nitrogen capture from a common soil pool, Such creative studies are even more needed to increase our understanding of the processes governing successional plant communities with more than two species. Labelling techniques using dual or triple tracers to obtain accurate information on these important processes may enable such studies under field or semi-field conditions. The combination of ^{14}C and ^{15}N will for example elucidate to what degree Nitrogen transfer is carbon-borne. The combination of multiple tracers and multiple species mixtures could also aid in understanding the mechanisms involved in the transfer process. The task of defining each nitrogen flux is however very difficult due to problems associated with pool mixing and pool dilutions in the donor plants, the receiver plant, as well as in the intermediate pools in the soil and soil solution.

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