VIEWPOINT

Ecological significance and complexity of N-source preference in plants

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Received: 29 April 2013 Returned for revision: 20 May 2013 Accepted: 29 May 2013

INTRODUCTION

Terrestrial plants take up inorganic nitrogen from the soil mainly in the forms of ammonium, NH$_4$$^+$ and nitrate, NO$_3$$^-$, two ions that, when acquired, have highly distinct genetic and metabolic consequences in the plant (Haynes and Goh, 1978; Britto and Kronzucker, 2002, 2005a; Stitt et al., 2002). Some plant species have been shown to produce more biomass, or accumulate greater quantities of nitrogen, when growing on one N source compared with another, i.e. they appear to display a preference. Despite the importance of N as a frequently growth-limiting nutrient in both agricultural and wild ecosystems (Tilman, 1985; Vitousek and Howarth, 1991), however, no precise definition of N-source preference (denoted here as $\beta$) after Boudsoq et al., 2012 has emerged in the literature, nor has a robust, broad classification of plant species adapted to NO$_3$ or NH$_4$ (although a few preliminary attempts have been made, e.g. Krajina et al., 1973; Falkengren-Gerup, 1995; Britto and Kronzucker, 2002). This is in part because the interactions between plant acquisition of NO$_3$ or NH$_4$ and multiple environmental variables, such as temperature, soil pH and nutrient supply, produce a complex of effects that can greatly influence and shift plant growth responses to variable N sources. In an ecological setting, this physiological complexity is compounded by the variability inherent in many ecosystems, where large changes in soil characteristics frequently occur over short distances and short spans of time (Hodge, 2004). In this paper we shall discuss the complexities involved in accurately modelling $\beta$ within ecological contexts, and the significance of such preferences for ecological processes such as succession.

PHYSIOLOGICAL UNDERPINNINGS OF N-SOURCE DIFFERENCES

The reasons underlying apparent N-source preferences are poorly understood, but include ammonium toxicity in nitrate specialists (Gerendás et al., 1997; Britto and Kronzucker, 2002) and atrophied nitrate uptake systems in the roots of ammonium specialists (Kronzucker et al., 1997). Fundamental questions remain unanswered in this branch of physiology, such as that of why many plants, especially agricultural crop species and early-successional pioneer species, appear to prefer NO$_3$ to NH$_4$, despite the fact that NO$_3$ must be taken up against a steep electrochemical gradient, then reduced to NH$_4$ before entering the organic N pool. Both processes require a considerable additional expenditure of fixed carbon compared with NH$_4$ acquisition (Bloom et al., 1992; Kurimoto et al., 2004; Britto and Kronzucker, 2005a). One answer to this appears to lie in the rapid entry of NH$_4$ into roots, which occurs even in NO$_3$ specialists, and can result in pronounced accumulation of NH$_4$ (Givan, 1979; Gerendás et al., 1997; Britto et al., 2001). Ammonium build-up can consequently have toxic effects, including the suppressed uptake of important cationic nutrients, such as K$^+$, Ca$^{2+}$ and Mg$^{2+}$ (Kirkby, 1968; Salsac et al., 1987; van Beusichem et al., 1988; Lewis, 1992; Britto and Kronzucker, 2002). By contrast, NO$_3$ toxicity is fairly uncommon and typically occurs at very much higher soil concentrations (Britto and Kronzucker, 2005b, and references therein). Among the strategies used by plants to decrease the amount of free NH$_4$ in tissues is increased NH$_4$ assimilation (Givan, 1979; Magalhaes et al., 1995; Gerendás et al., 1997; Schortemeyer et al., 1997). However, this requires an elevated supply of carbohydrate to the roots, because only a small amount of NH$_4$ is translocated to shoots (Wang et al., 1993; Kronzucker et al., 1998; Finnemann and Schjoerring, 1999), leaving less reduced carbon available for growth and maintenance (Lewis, 1992). Another strategy is to increase the efflux of NH$_4$ to the external medium, which can, however, result in an energetically costly futile cycle (Britto et al., 2001; Li et al., 2012). Energy lost in this cycle, in addition to the frequently suggested uncoupling of energy gradients in cellular organelles by NH$_4$ (Krogmann et al., 1959; Crofts, 1967; this possibility is disputed, however – see Gerendás et al., 1997;
Britto and Kronzucker, 2002), may eliminate any energetic advantages conferred by the uptake of reduced N. Although elevated respiration under high-NH$_4^+$ conditions has been attributed to futile NH$_4^+$ cycling in roots (Britto et al., 2001), the link between carbohydrate limitation and preference against NH$_4^+$ is not straightforward, since increased light intensity may aggravate rather than alleviate NH$_4^+$ toxicity (Gerendás et al., 1997; Zhu et al., 2000).

Deficiency, sufficiency and toxicity occur for both inorganic N forms, with very different set points, and growth optima are expected and commonly observed (Fig. 1; Gray, 1983; Eck, 1984; Westfall et al., 1990; Miller and Timmer, 1994; Padgett and Allen, 1999; Cabrera, 2000; Gan et al., 2012; Hall, 2002). Such optimum curves, and how they differ with N source for a given plant species, should inform any fundamental appraisal of the N-source preference(s) of that species, since they are directly linked with plant productivity. Root transport systems specific for NO$_3^-$ and NH$_4^+$ acquisition fundamentally influence the shapes of growth–response curves, since their activities not only determine the amount of excessive, detrimental transport that may occur at high substrate concentrations (Britto et al., 2001) but also govern the lowest soil N concentration from which a species can abstract the limiting resource and at which it can survive. This concentration minimum is frequently referred to as the C$_{\text{min}}$ and may be as low as ~0.001 mm for both NO$_3^-$ and NH$_4^+$ (Deane-Drummond and Chaffey, 1985; Marschner et al., 1991). The C$_{\text{min}}$ concept is important in modelling processes such as competition and succession, and is similar to important ecological principles such as Justus von Liebig’s ‘law of the minimum’, and Tilman’s R* rule, which predicts the outcome of competitive interactions between species based on their ability to survive on the lowest amount of a limiting resource (Tilman, 1982; McGill 2005; Wilson et al., 2007).

However, it should be emphasized here that C$_{\text{min}}$ (like β, as will be discussed) is not constant for a plant species, but varies with root activity, which in turn strongly depends on factors such as temperature and plant nutrient status (Drew et al., 1984; Marschner et al., 1991).

Because the regulation of inorganic N transport largely determines the uptake of nutrients at both limiting and toxic concentrations, it is crucial to examine it in the present context. Particularly important is the physiological principle that the transport systems moving inorganic N across root plasma membranes, as well as the metabolic systems assimilating N within the cell, are typically downregulated by increasing soil nutrient content, especially that of N itself (Glass et al., 2002; Tang et al., 2012). The curves in Fig. 2 exemplify this pattern of regulatory feedback by long-term N supply and plant N status on the kinetics of N uptake (Wang et al., 1993; Rawat et al., 1999; Kronzucker et al., 2000). Similar patterns are seen for both NO$_3^-$ and NH$_4^+$, but with unique kinetic parameters for each. It is important to note that these curves are typically hyperbolic in shape, as in Monod or Michaelis–Menten equations, under most ecologically relevant conditions; in other words, they saturate, as does the growth response to increasing soil N. Under toxicity or ‘luxury consumption’ conditions, however, linearly rising patterns of unidirectional influx are sometimes observed, but elevated influx is largely compensated for by elevated efflux under these conditions (Britto and Kronzucker, 2006) and does not translate into increased growth (Gezelius and Näsholm, 1993; de Mazancourt et al., 2012).

An added layer of regulatory complexity can be seen in the case of the nitrate acquisition apparatus, which is greatly downregulated in the absence of NO$_3^-$ (Kronzucker et al., 1999a; Glass et al., 2001). The induction of this apparatus by the appearance of nitrate can be quite rapid in some species, taking only a few hours (Siddiqi et al., 1989; Kronzucker et al., 2000), while in others, such as white spruce, it can take several days (Kronzucker et al., 1995; Min et al., 1998). Once stimulated, NO$_3^-$ transport is usually downregulated by further NO$_3^-$ acquisition, but this is not well understood; there appear to be species-specific differences in the metabolites responsible for this effect (Glass et al., 2002; Tang et al., 2012).

Regardless of the mechanism, it is well known that changes in soil N pools can exert powerful influences on N-acquisition characteristics, and bring changes in apparent N-source preferences, even at sub-toxic concentrations (Tylova-Munzarova et al., 2005; Munzarova et al., 2006; Houlton et al., 2007).

Many other environmental factors influence the uptake of N, with divergent effects found for NO$_3^-$ and NH$_4^+$. One of the most important is soil temperature (Haynes and Goh, 1978; Sasakiwa and Yamamoto, 1978; Clarkson and Warner, 1979; Kafka, 1990; Macduff and Jackson, 1991; Gessler et al., 1998; Vaast et al., 1998; Kumar et al., 2008), which can fluctuate greatly on time scales of months, days or hours. In general, NO$_3^-$ uptake appears to be more inhibited by low temperature than does NH$_4^+$ uptake (Frota and Tucker, 1972; Clarkson et al., 1994), which can be explained by its higher energy requirement for
acquisition and reduction (see above). Soil pH also shows high variability (Farley and Fitter, 1999) and affects $\text{NO}_3^{-}$ and $\text{NH}_4^{+}$ transport differentially, with optima tending to be more alkaline for $\text{NO}_3^{-}$ uptake than for $\text{NH}_4^{+}$ uptake (Haynes and Goh, 1978; von Wirén et al., 1997; Hawkins and Robbins, 2010). This is somewhat surprising given that there is a higher proton requirement for $\text{NO}_3^{-}$ uptake than for $\text{NH}_4^{+}$, since it is driven by a proton-symport mechanism (McClure et al., 1990; Britto and Kronzucker, 2006; Millet et al., 2007), while $\text{NH}_4^{+}$ uptake probably occurs in a uniport manner, in exchange for protons (though not directly coupled to $H^+$ transport), under most conditions (Ludewig et al., 2002; Mayer et al., 2006; at very low concentrations, $\text{NH}_4^{+}$ uptake involves a symport mechanism with protons (Ortiz-Ramirez et al., 2011), while at high concentrations $\text{NH}_3$ permeation may be possible through aquaporins (Jahn et al., 2004)). The mechanisms of transport for the two ions themselves have distinct consequences for soil pH: alkalization by $\text{NO}_3^{-}$ nutrition and acidification by $\text{NH}_4^{+}$ nutrition (Raven and Smith, 1976; Runge, 1983; van Beuschem et al., 1988; Marschner et al., 1991; Falkengren-Gruner, 1995; Britto and Kronzucker, 2002). These processes can result in unpredictable feedback cycles that can be intensified by other factors, such as (1) inhibition of nitrification rates by soil bacteria as the rhizosphere acidifies (Haynes and Goh, 1978; Falkengren-Gruner, 1995) and (2) changes in the availability of nutrients, such as $P$, or toxicants, such as $Al$, as soil pH changes differentially depending on which N source dominates in plant acquisition patterns (Riley and Barber, 1971; Ruan et al., 2000).

Other major components of plant nutrition interact profoundly with plant roots to produce divergent effects when grown on $\text{NO}_3^{-}$ or $\text{NH}_4^{+}$. One of the most notable examples of this is the alleviation of $\text{NH}_4^{+}$ toxicity by $K^+$ supply (Mengel et al., 1976; Santa-Maria et al., 2000; Roosta and Schjoerring, 2008; Balkos et al., 2010; ten Hoopen et al., 2010; Li et al., 2012). Another lies in the interactions between $\text{NH}_4^{+}$ and $\text{NO}_3^{-}$ themselves, as the significant, and variable, inhibition of $\text{NO}_3^{-}$ uptake by $\text{NH}_4^{+}$ (Minotti et al., 1969; Lee and Drew, 1986; Marschner et al., 1991; Kreuzwieser et al., 1997; Kronzucker et al., 1999a) and the synergistic effect on N uptake and growth often seen when the two N sources are combined (Cox and Reisenauer, 1973; Kronzucker et al., 1999a, b). A third important example is seen in the strong interactions between carbohydrate supply and growth on different N sources (Haynes and Goh, 1978; Aslam et al., 1979; Givan, 1979; Kafka, 1990), while a fourth is that between inorganic and organic N. Regarding this last example, inorganic vs. organic, the soil N content in some ecosystems, such as boreal forests, can be predominantly in the form of amino acids, which can be taken up by roots in substantial quantities (Nåsholm et al., 1998; Lipson and Nåsholm, 2001). This has significance in terms of providing an alternative source of N when this resource is limiting, thus reducing plant demand for $\text{NO}_3^{-}$ and $\text{NH}_4^{+}$. Moreover, the downregulatory effect of amino acids on the uptake of $\text{NO}_3^{-}$ and $\text{NH}_4^{+}$ is well known (Imsande and Touraine, 1994; Padgett and Leonard, 1996; Rawat et al., 1999; Gessler et al., 2004).

Further complex nutritional interactions of broad significance should be mentioned here, and can be considered in three groups. The first group consists of interactions among N source, soil moisture and water use. For instance, increased drought adaptation has been observed with $\text{NH}_4^{+}$ nutrition in some cereals (Mihailović et al., 1992; Yin and Raven, 1998; Guo et al., 2008), while other studies, mainly in dicotyledons, have shown decreased water-use efficiency under $\text{NH}_4^{+}$ (Raven et al., 1992; Hög-Jensen and Schjoerring, 1997; Claussen, 2002; Lu et al., 2005). Moreover, in a striking study of N-source preference among a functionally diverse group of tropical forest species, Houlton et al. (2007) showed abrupt community-wide changes in $\beta$ resulting from changes in precipitation. These authors found that $\text{NO}_3^{-}$ was the preferred source for all species under dry conditions, while in the wettest soils $\text{NH}_4^{+}$ was almost exclusively used.

The second group of interactions are those between N source and light intensity. Light plays a key role in regulating key components of the $\text{NO}_3^{-}$-acquisition pathway (Stitt et al., 2002), while $\text{NH}_4^{+}$-grown plants tend to be more sensitive to light stress than $\text{NO}_3^{-}$-grown plants (Magalhaes and Wilcox, 1983; Zornoza et al., 1987; Zhu et al., 2000).

The third group of interactions are those between N source and changes in atmospheric $\text{CO}_2$: this topic, of considerable recent interest, has yielded mixed results, but elevated $\text{CO}_2$ can produce considerable differential effects on the acquisition of $\text{NO}_3^{-}$ and $\text{NH}_4^{+}$, at times causing switches in plant preference (Bassirirad et al., 1996, 1997; Zerihun and Bassirirad, 2001).

The plasticity of plant responses to environmental variability reflects the complexity inherent in all ecosystems. This is particularly true in the case of the multifarious environmental factors influencing $\beta$, which must be included when comprehensively modelling the ecological consequences of such preferences. For example, even a seemingly simple agricultural system such as a rice paddy can be dauntingly complex in terms of modelling nitrification and nitrate use (Kirk and Kronzucker, 2005), since these activities, and the resulting magnitudes of N pools, can vary substantially over temporal and spatial scales. Indeed, the modelling of nitrification alone can require the consideration of as many as nine independent processes (Vitousek and Melillo, 1979). Moreover, the horizontal and vertical patchiness of soils with respect to water and nutrients is well known (Hodge, 2004); the variability of resources can be as great over the rooting zone of a plant as it is over a 120-m$^2$ plot (Jackson and Caldwell, 1993). Such patchiness extends to substantial, and unpredictable, temporal variations in nutrient availability, including that of $\text{NH}_4^{+}$ and $\text{NO}_3^{-}$ (Drew and Saker, 1975; Farley and Fitter, 1999). An additional layer of complexity arises from the ability of vegetation to strongly influence the nutrient composition of soils; in one study, 10-fold variation in net mineralization was observed within 3 years in initially identical soils, as a result of the presence of different plant species (Wedin and Tilman, 1990). In all these considerations, it is important to understand that nutrient pool sizes do not necessarily reflect the importance of a pool in an ecosystem. While some pools may be small, this may simply be due to the very rapid turnover caused by intensive utilization and replenishment of the pool (Robertson and Vitousek, 1981; Schulze et al., 1994; Evine and Chapin, 1997; Hart and Stark, 1997; Kirk and Kronzucker, 2005).

Taken together, the above considerations strongly indicate that the concept of N-source preference cannot be easily defined, but depends on a wide and dynamic range of environmental and physiological factors that overlap simultaneously. Strictly speaking, any assignment of a rigid preference index is valid only for the composite of conditions under which it was experimentally
determined. Moreover, it may be valid only for a given plant variety or subspecies (e.g. Zornoza et al., 1996) or developmental stage (Haynes and Goh, 1978; Jing et al., 2012).

MODELLING THE INFLUENCE OF N-SOURCE PREFERENCE ON ECOLOGICAL PROCESSES

Despite the complex physiological nature of β values, plants have certainly evolved nutritional adaptations to NO$_3^-$ or NH$_4^+$, and thus can often be found on soils enriched in the particular N source to which they are most adapted for a given condition. Indeed, some plants appear to be so well adapted to a specific N source that they appear to prefer it under a wide range of conditions. For example, in one study seedlings of Picea glauca and Pinus radiata showed greater growth and N uptake with NH$_4^+$ than with NO$_3^-$, regardless of pH, temperature or type of growth medium (McFee and Stone, 1968). The slow growth of these late-successional conifers on NO$_3^-$ may be particularly limited due to highly atrophied transport systems for the ion, as has been demonstrated in P. glauca (Kronzucker et al., 1997), while their superior growth on NH$_4^+$ reflects the high NH$_4^+$ content and low nitrification potential of boreal forest soils and other climax systems (Rice and Pancholy, 1972; Haynes and Goh, 1978). Other studies have indicated at times extraordinary plant preferences for one inorganic N source over another, at various developmental stages and under differing nutritional conditions (Rygiewicz and Bledsoe, 1986; Knoepp et al., 1993; van den Driessche and Ponsford, 1995; Gessler et al., 1998).

In such species and under such conditions, where N-source preferences appear to be pronounced, soil nitrogen speciation (and changes in it) has been shown to be a significant determinant of plant productivity, competition, coexistence and ecological succession (McFee and Stone, 1968; Rice and Pancholy, 1972; Haynes and Goh, 1978; Lodhi, 1979; Lodhi and Killingbeck, 1980; Robertson and Vitousek, 1981; Kronzucker et al., 1997, 2003; Kirk and Kronzucker, 2005; Kahmen et al., 2008). Such work extends to interactions among plant species and soil microorganisms in terms of their competition, cooperation and N-source preferences, which can change depending on the presence or absence of mycorrhizal associations (Plaassard et al., 1991; Stewart et al., 1993; Clemmensen et al., 2008; Warren, 2009; Paulding et al., 2010; Piao et al., 2012; Wu et al., 2013).

Nevertheless, many other plants have eluded simple classification as preferring NO$_3^-$ or NH$_4^+$. For example, some apparent NO$_3^-$ specialists can thrive on NH$_4^+$ when K$^+$ provision is sufficiently high (Britto and Kronzucker, 2002). Most strikingly in this regard, a quintessentially ‘NH$_4^+$-preferring’ plant such as rice (Oryza sativa) can show significant variations in its apparent β, depending on factors such as soil depth and nutritional composition, and can be very effective in NO$_3^-$ utilization (Kronzucker et al., 2000; Kirk and Kronzucker, 2005; Balkos et al., 2010). Another such example is that of Vaccinium species, which are often considered to be NH$_4^+$ specialists (Claussen and Lenz, 1999; Britto and Kronzucker, 2002), but in some studies the co-presence of NO$_3^-$ and NH$_4^+$ appears to be preferable (Ingestad, 1973). Thus, the development of a general model to predict the effect of β on ecological outcomes (e.g. of competition) should assume the multifactorial nature of N-source preference or, more precisely, a set of preferences rather than a fixed singularity.

The sheer complexity of possible plant responses to inorganic N source is a major reason why no comprehensive models yet exist to map and predict the effects of β on ecological processes. Recently, an attempt was made to model species replacement dynamics and ecosystem-level plant productivity, using a measure of N-source preference (Boudsocq et al., 2012). Unfortunately, a very simple designation of β was used in the process; it was defined as a linear coefficient for NH$_4^+$ uptake, ranging from 0 to 1, while (1 − β) was assigned to be the corresponding coefficient for NO$_3^-$ uptake. A fixed N-source preference was thereby assumed for a given plant species, as was a linear proportionality between uptake and substrate concentrations. As discussed here, however, a fixed β value does not take into account the genetic and physiological plasticities of plant responses to NO$_3^-$ or NH$_4^+$ within the matrix of changing environmental conditions.

FIG. 3. Physiological and environmental factors influencing N-source preferences (β values) in plants.
(e.g. Fig. 2). In addition, this definition neither considers Monod-like (or Michaelis–Menten-like) growth and uptake patterns nor allows for luxury or toxic consumption (Fig. 1).

For purposes of ecosystem management and study, it is worthwhile to develop general models that describe and predict effects of N source on plant populations and their competitive interactions. What must be kept in mind is that pronounced differences exist in plant responses to NO$_3^-$ or NH$_4^+$, but how these translate into ‘preferences’ depends on many complex, and interacting, factors. Indeed, a simple assessment of $\beta$ is not generally feasible or applicable to realistic models, despite recent effort to do so (Boudsooq et al., 2012). The numerous factors, physiological and environmental, that coalesce to influence N-source preference are outlined in Fig. 3; each has its own set of complexities and interactions with other factors. In some cases, such as that of many boreal conifers, a strong specialization emerges despite myriad complexities (but cf. Heiskanen, 2005), while in cases like tropical lowland rice apparent preferences are not so clear-cut.

To conclude, a quotation from D. Tilman (1985) is instructive: ‘Clearly, the uniqueness of the species involved in successions in different areas, the uniqueness of each particular habitat, and various historical factors all limit the potential predictive ability of any model of vegetation dynamics and structure’.

ACKNOWLEDGEMENTS

We thank the Natural Sciences and Engineering Council of Canada (NSERC; grant # 217277-2009) and the Canada Research Chairs programme (CRC) for supporting this work.

LITERATURE CITED


