

## Original Article

## The carbon bonus of organic nitrogen enhances nitrogen use efficiency of plants

Oskar Franklin<sup>1,3</sup>, Camila Aguetoni Cambui<sup>2</sup>, Linda Gruffman<sup>3</sup>, Sari Palmroth<sup>4</sup>, Ram Oren<sup>4</sup> & Torgny Näsholm<sup>2,3</sup>

<sup>1</sup>Ecosystems Services and Management Program, International Institute for Applied Systems Analysis, A-2361 Laxenburg, Austria, <sup>2</sup>Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, SE-901 85 Umeå, Sweden, <sup>3</sup>Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden and <sup>4</sup>Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA

## ABSTRACT

**The importance of organic nitrogen (N) for plant nutrition and productivity is increasingly being recognized. Here we show that it is not only the availability in the soil that matters, but also the effects on plant growth. The chemical form of N taken up, whether inorganic (such as nitrate) or organic (such as amino acids), may significantly influence plant shoot and root growth, and nitrogen use efficiency (NUE). We analysed these effects by synthesizing results from multiple laboratory experiments on small seedlings (Arabidopsis, poplar, pine and spruce) based on a tractable plant growth model. A key point is that the carbon cost of assimilating organic N into proteins is lower than that of inorganic N, mainly because of its carbon content. This carbon bonus makes it more beneficial for plants to take up organic than inorganic N, even when its availability to the roots is much lower – up to 70% lower for Arabidopsis seedlings. At equal growth rate, root:shoot ratio was up to three times higher and nitrogen productivity up to 20% higher for organic than inorganic N, which both are factors that may contribute to higher NUE in crop production.**

*Key-words:* allocation; amino acids; ammonium; assimilation; growth model; nitrate; nitrogen source; plant theory.

## INTRODUCTION

While traditionally inorganic nitrogen (iN) has been viewed as the dominant N source for plants, the importance of organic N (oN) is now widely recognized. Organic and inorganic N forms coexist in soil and represent different stages in the N transformation processes. Because in soil oN is a precursor of iN it may be competitively advantageous for plants to preferentially take up oN, which has been observed in N limited tundra plants (Chapin *et al.* 1993) and wheat (Geisseler *et al.* 2009). With the exception of the initial period after N fertilizer application when iN concentrations are elevated, oN in the form of amino acids represent a significant proportion of exchangeable and soluble N pools in agricultural soils (Brackin *et al.* 2015; Holst *et al.* 2012; Jämtgård *et al.* 2010) and dominate in the organic layer of forest

soils (Inselbacher & Näsholm 2012). Peptides (Hill *et al.* 2011; Schmidt *et al.* 2003) and proteins (Paungfoo-Lonhienne *et al.* 2008) also represent important oN sources. Even if the relative contribution of amino acids to the N budget of crops remains uncertain, all plants studied so far have the capacity to acquire and metabolize amino acids, and all soils studied in this respect contain amino acids (Paungfoo-Lonhienne *et al.* 2012).

Importantly, the effects of different N forms are interesting not only from a pure scientific perspective, but also for agriculture, in particular the potential of organic N to enhance N use efficiency (NUE) (Paungfoo-Lonhienne *et al.* 2012). Although oN may increase NUE compared to inorganic N fertilizers, as shown for urea (Arkoun *et al.* 2012), most agricultural practices rely on inorganic fertilizers. As currently more than half of the N added to cropland is lost to the environment, producing threats to air, water, soil and biodiversity, improving NUE is of global importance (Lassaletta *et al.* 2014). Recent measurements of N availability at the scale of roots (rather than bulk soil) in a sugarcane plantation showed that plants were not able to fully capitalize the very high iN concentration in the soil after fertilization, but that they took up N efficiently under conditions prevailing between fertilization events, mainly in the form of oN (amino acids) (Brackin *et al.* 2015). This suggests a potentially important role of oN for improving NUE in agriculture – a role which is however not well understood in terms of the further growth effects on the plants.

The effects of N form on plants have been mainly studied from an availability perspective. Many studies have addressed the preferences and partitioning of N forms among plants and, although results are variable (Andersen & Turner 2013; Ashton *et al.* 2008; Harrison *et al.* 2007; Harrison *et al.* 2008; McKane *et al.* 2002; Miller & Bowman 2002; Miller *et al.* 2007; Schimel & Chapin 1996; Wei *et al.* 2015; Wilkinson *et al.* 2015), it seems reasonable that plants are adapted, or acclimate, to preferentially take up the N form most available to them (Boczulak *et al.* 2014; Scott & Rothstein 2011). However, to better understand the role of N form for plant performance, it is necessary to know not only the availability of different N forms, but also what the benefits and costs of their uptake are for the plant. Does it matter to the plant if the N taken up is iN or oN?

Because soil microbes may convert N between different forms, it is difficult to experimentally investigate the effects of N form on

Correspondence: O. Franklin. Fax: +432236807599 Email: franklin@iiasa.ac.at

plants in the field, and the results of such experiments may be unreliable. In sterile laboratory experiments, plants supplied with oN showed different root morphology and higher root:shoot ratio than those supplied with iN, even though both N forms were supplied at the same N concentration and plants had similar internal N concentration (Cambui *et al.* 2011; Lonhienne *et al.* 2014). Potential underlying physiological mechanisms have been identified, including different assimilation sites of iN and oN (Cambui *et al.* 2011), and differences in assimilation costs (De Vries *et al.* 1974), which were quantified in the seminal work of Zerihun *et al.* (1998). However, the ultimate effects on whole plant growth and allocation of oN versus iN are not well understood. Whereas bottom-up biochemical calculations suggest that the difference in assimilation costs between N forms is too small to influence growth and allocation (Zerihun *et al.* 1998), experimental studies show large effects on allocation (Cambui *et al.* 2011). These contrasting results may reflect differences among studies in growth conditions, such as soil N availability, plant size and light level, which interact with the biochemical effects of N form. For example, while oN have energetic assimilation advantages over iN (De Vries *et al.* 1974; Gruffman *et al.* 2013; Zerihun *et al.* 1998), it may require higher root investments for uptake, leading to reduced shoot:root ratio and, in-turn, reduced light capture. To understand such potentially complex interactions, we need to understand how they are coordinated; in effect, an organizing principle for plant behaviour is required.

Plant behaviour is ultimately the result of evolution towards increasing fitness (reproductive production per capita), accounting for growth, survival and reproduction over the lifetime of individuals. However, a particular life-stage fitness can be approximated well by a simpler goal function (Dewar *et al.* 2009; Franklin *et al.* 2012). Small, young plants are expected to allocate C and N among organs to maximize relative growth rate (Ågren & Franklin 2003), a principle allowing us to construct a tractable model of plant growth and its response to N and light availability. Here we describe the model and use it to interpret the results of multiple laboratory experiments on the effects of N availability in different forms (nitrate, ammonium and amino acids) on plant growth, allocation and biomass N concentration. We find that the observed effects of N form are explainable based on two primary factors: N assimilation costs and N uptake per root

mass. Specifically, compared to iN, growth on oN alone or in combination with iN leads to (1) lower N assimilation cost and (2) lower N uptake per root mass, i.e. higher root C costs per N taken up. We then used the model to answer the questions: How does the benefit of a lower N assimilation cost add up with its higher uptake cost, i.e. under which soil N conditions is the net growth effect positive for the plant?, and what are the consequences for N use efficiency?

## METHODS

### Experiments

The experimental methods used for *Arabidopsis thaliana* (*Arabidopsis*) are fully described by Cambui *et al.* (2011) and also apply to the *Populus tremula* (poplar) experiment. Here we provide a brief summary of the methods. Wild type *Arabidopsis* (ecotype Col-0) were grown on sterile agar plates which were incubated in a cold room for two days to synchronize germination and then transferred to a growth cabinet with a 16/8 light/dark ( $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) light regime. Nitrogen was administered according to Table 1. We used arginine and glutamine as organic N forms because they are abundant in soils and also that they represent basic and neutral amino acids, respectively. Plant shoots were not in contact with the agar surface, so all N in plants was derived from root uptake. Poplar plants (*P. tremula*) were grown from seeds of local origin under identical conditions as *Arabidopsis* with the exception that the growth units consisted of plastic boxes (filled with 100 mL agar; one seed per box) and plants were harvested after 28 days. At harvest, roots and shoots were dried and weighed, and N and C concentrations were analysed with an elemental analyser (Flash EA 1112, Thermo Fisher Scientific).

Scots pine and Norway spruce seedlings were cultivated in green house conditions,  $200\text{--}300 \mu\text{mol m}^{-2} \text{s}^{-1}$  constant light, with pine seeds originating from seed orchard Våge 125 (mean clonal origin:  $65^{\circ}48'N$ , 440 m.a.s.l.) and spruce seeds originating from seed orchard Domsjöänget 130 (mean clonal origin:  $64^{\circ}24'N$  375 m.a.s.l.). Seedlings were grown in 0.5 L pots containing unfertilized peat. Four seedlings were grown in each pot and fertilized with 80 mL ( $20 \text{ mL seedling}^{-1}$ ) of chosen N form and concentration twice a week (Table 1) and additional

**Table 1.** Experiments

Experiment	Plant species	No. plants per treatment	Inorganic N form (iN)*	Mixed N form (ioN)*	Organic N form (oN)*	Growth substrate
Arab 1	<i>Arabidopsis</i>	10	$\text{NO}_3 + \text{NH}_4$ [6]	$\text{NO}_3 + \text{Gln}$ [6]		Agar plates
Arab 2	<i>Arabidopsis</i>	10	$\text{NO}_3$ [3]		Gln [3]	Agar plates
Pop	<i>Populus tremula</i>	8 (oN), 10 (iN)	$\text{NO}_3 + \text{NH}_4$ [6]	$\text{NO}_3 + \text{Gln}$ [6]		Agar plates
Pine	<i>Pinus sylvestris</i>	16	$\text{NO}_3 + \text{NH}_4$ [1, 3]	$\text{NO}_3 + \text{Arg}$ [0.75, 2.25]	Arg [1, 3]	Pots
Spruce	<i>Picea abies</i>	16	$\text{NO}_3 + \text{NH}_4$ [1, 3]	$\text{NO}_3 + \text{Arg}$ [0.75, 2.25]	Arg [1, 3]	Pots

\*For Arab 1, Arab 2 and Pop numbers in []-brackets are substrate N concentrations given in mM N and for pine and spruce they refer to N concentrations of added fertilizer.

watering was carried out once a week. Eight pots from each treatment and concentration were harvested after 90 days and analysed for morphological characteristics. Each sample contained four seedlings which were pooled. Seedlings were dried at 60 °C for 72 h. Needles were milled into a fine powder in a bead mill and thereafter analysed with an elemental analyser Flash EA 1112, Thermo Fisher Scientific, to determine the nitrogen content.

For each sample we calculated relative growth rate from biomass, growing time, and initial biomass or seed weight, based on the assumption that relative growth rate was constant over time (see Theory and model). Root N acquisition ( $u$  in the model) was calculated as total plant N/root mass. In addition we calculated N productivity (the growth rate per plant N; Ågren & Bosatta 1998), as relative growth rate/biomass N concentration.

### Theory and model

We constructed a mechanistic model of plant growth applicable to small plants and seedlings, which are assumed to allocate C and N among organs to maximize relative growth rate (Ågren & Franklin 2003). The use of small plants in laboratory environment not only has practical experimental advantages, but also makes the interpretation of the results simpler by minimizing the impact of non-measured and uncontrolled factors. The small plants also make modeling more accurate for the same reason, and allowed us to use the assumptions that the plants are growing exponentially (relative growth rate is constant) without interference among roots and leaves (no competition among roots or self-shading among leaves), and that N concentration and root:shoot ratio stay constant during growth. Exponential growth and constant N concentration were confirmed for our growing conditions based on an experiment similar to our Arab 1 experiment (Fig. S1). Although relative growth rate eventually declines as plants get larger, it is normally constant during the initial phase of growth which we analyse in this study (Ågren & Bosatta 1998; Lambers *et al.* 1998; Paine *et al.* 2012).

Plant productivity is modelled as C gain – a function of shoot photosynthetic rate ( $A$ , gC g<sup>-1</sup> d<sup>-1</sup>) times fraction shoot biomass ( $f_s$ ) – minus C losses because of biomass construction costs (growth respiration) and maintenance respiration ( $r_m$ , gC gN<sup>-1</sup> d<sup>-1</sup>), which is proportional to plant N content ( $N$ ; Ryan *et al.* 1996). The effect of growth respiration is often expressed in term of a general conversion efficiency from C gain to biomass ( $y_0 \approx 0.7$ ; Choudhury 2001). However, we explicitly model the costs of assimilating N into biomass ( $r_A$ , gC gN<sup>-1</sup>), which is part of the overall growth respiration. While we do not have an estimate of the non-N dependent part of the growth respiration, we know the total biomass construction costs in terms of  $y_0$ . Thus, to account for the fact that the N assimilation cost is also a part of the overall growth respiration, we reduce the overall growth respiration to compensate for double counting (Supporting methods). This reduction results in an adjusted (increased) growth efficiency of  $y=1.0$  for Arabidopsis and poplar plants and  $y=0.82$  for pine and spruce. Combining the above assumptions for C gain and C costs leads to the following equation for biomass ( $B$ ) growth:

$$\frac{dB}{dt} = \left[ A f_s B - \frac{dN}{dt} r_A - r_m N \right] \frac{y}{f_C}. \quad (1)$$

In Eqn 1,  $f_C$  is biomass carbon concentration (in gC g<sup>-1</sup>).

Nitrogen is taken up in proportion to root growth and root N acquisition (N uptake per root biomass,  $u$ , gN g root<sup>-1</sup>):

$$\frac{dN}{dt} = u (1 - f_s) \frac{dB}{dt}. \quad (2)$$

Alternatively, N uptake could be modelled as a function of root mass, i.e. integrated growth instead of growth. However, because we are modelling exponential growth this (integration of an exponential function) would only lead to quantitative but no qualitative change in the relationship between  $B$  and N uptake.

Under constant relative growth rate, the relative change of plant N content and biomass must be equal:

$$\frac{1}{N} \frac{dN}{dt} = \frac{1}{B} \frac{dB}{dt} \quad (3)$$

From Eqns 2 and 3 follows that  $f_s$  is a function of N concentration ( $c$ ) and  $u$ :

$$f_s = 1 - \frac{c}{u}. \quad (4)$$

We now derive an expression for the relative growth rate by inserting Eqns 2, 3 in Eqn 1:

$$\frac{1}{B} \frac{dB}{dt} = [A f_s - r_m c] \frac{1}{\frac{f_C}{y} + r_A c}. \quad (5)$$

Carbon assimilation ( $A$ , Eqn 6) was modelled as done in Franklin (2007) based on day length ( $f_d$ , h d<sup>-1</sup>) and the non-rectangular hyperbola model of photosynthesis (Thornley 1998), co-limited by N concentration ( $c$ , which reflects the protein content in metabolic machinery) and light intensity. N concentration and its minimum value ( $c_0$ ) control light saturated photosynthetic capacity via the parameter  $a$  (gC gN<sup>-1</sup> h<sup>-1</sup>). Incident light intensity determines potential light limited photosynthesis ( $Q$ , gC g<sup>-1</sup> h<sup>-1</sup>). The curvature parameter  $\theta$  controls the transition between light and N limitation.

$$A = \frac{f_d}{2\theta} \left[ Q + (c - c_0)a - \sqrt{(Q + (c - c_0)a)^2 - 4Qa\theta(c - c_0)} \right] \quad (6)$$

The expressions for  $A$  (Eqn 6) and  $f_s$  (Eqn 4) are inserted in Eqn 5 to get relative growth rate as a function of biomass N concentration ( $c$ ). We assume that the plant acclimates to its environment (defined by N availability, N form taken up and light environment) by optimizing N concentration to maximize relative growth rate, i.e.  $\frac{\partial}{\partial c} \left( \frac{1}{B} \frac{dB}{dt} \right) = 0$ . Relative growth rate has a maximum with respect to  $c$  because it is subject to a trade-off

between C assimilation and C loss by respiration, which both increase with  $c$ , which in turn is also constrained by root N acquisition ( $u$ , Eqn 4) as illustrated in Fig. S2.

### Effects of N form taken up

N form enters the model via primary effects on two properties: (i) Carbon costs of N assimilation ( $r_A$ , gC gN<sup>-1</sup>), which depends on the energy and stoichiometric requirements for protein construction, and (ii) root N acquisition ( $u$ , gN g root<sup>-1</sup>), which depends on both soil N availability and the properties of roots.

Assimilation of N into proteins involves multiple biochemical processes consuming energy and C, which can be expressed in terms of glucose use (Zerihun *et al.* 1998). For uptake of NO<sub>3</sub> and NH<sub>4</sub> key processes are: Reduction into NH<sub>3</sub>, pH regulation, synthesis of glutamine (Gln), and synthesis of other amino acids and proteins from Gln. In comparison to iN, uptake of oN (e.g. Gln) saves energy and C otherwise used for reduction and Gln synthesis. The C gained from root-absorbed Gln may be incorporated into the plant biomass or used in the plant's energy metabolism or both. In an experiment identical to Arab 1 in this study, 40–48% of the C gained from Gln absorption was retained in the plant and thus 52–60% used in energy metabolism (Ganeteg *et al.* unpublished results). These benefits result in a net C gain of using oN relative to iN – a C bonus. The C bonus can be calculated from the total glucose required for assimilation of each N form (Zerihun *et al.* 1998) expressed as C cost, which are (in gC gN<sup>-1</sup>): NO<sub>3</sub> = 5.81, NH<sub>4</sub> = 4.32, glutamine = 2.16, arginine = 3.02 (Table 2). We evaluated the model based on these biochemical estimates, but also let the model generate the most likely estimates indirectly, based on the whole plant data from our growth experiments (see Model evaluation).

While we do not model the underlying factors and mechanisms of root N acquisition ( $u$ ), they may depend on characteristics of both root uptake and of the fluxes of different N compounds in the soil. A difference in total N uptake per root biomass between iN and oN may therefore result from either or both of root and soil characteristics. Studies comparing uptake rates of oN and iN show high rates of uptake of iN in the form of ammonium but low rates of uptake for nitrate. For oN, in the form of amino acids, uptake rates often fall in between the two major iN forms (Näsholm *et al.* 2009). Movement

**Table 2.** Biochemically calculated assimilation costs for different N sources in gC gN<sup>-1</sup> according to Zerihun *et al.* (1998)

N source	Gross C costs	Net N assimilation	
		C bonus <sup>a</sup>	C cost
NO <sub>3</sub>	5.81	0	5.81
NH <sub>4</sub>	4.32	0	4.32
Gln	4.30	2.14	2.16
Arg	4.30 <sup>b</sup>	1.29	3.02 <sup>b</sup>

<sup>a</sup>C bonus is equal to the molecular gC per gN.

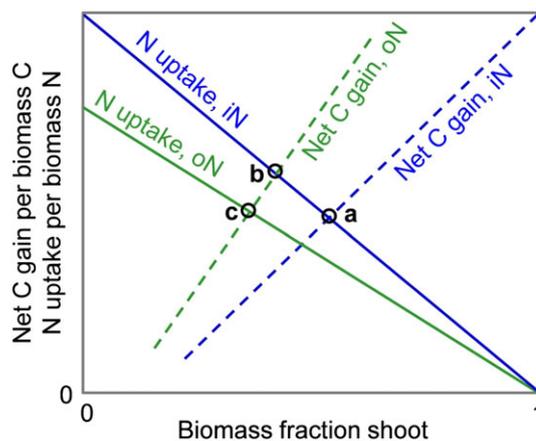
<sup>b</sup>Calculated assuming gross C costs (without C bonus) for N assimilation are equal to Gln.

of N compounds in the soil is in the form of diffusive flow and mass flow and most oN compounds would have lower flux rates in soil both because of their generally higher molecular weights and because of charge interactions with the solid soil phase (Jones *et al.* 2005; Owen & Jones 2001). However, regardless of the underlying mechanisms, the resulting N uptake per root biomass ( $u$ ) is what matters in our whole-plant model. We calculated this variable directly from measured root growth and N uptake.

Considering the requirement to balance the C and N economy of the plant we can illustrate the interaction of the two primary N-form effects, the C bonus and the root N acquisition, in a simple diagram (Fig. 1). Based on this graphical analysis, going from iN to oN nutrition, we should expect either an increase or decrease in growth rate, but always an increase in fraction root biomass (and reduced shoot fraction).

### Model evaluation

We used a Markov chain Monte Carlo method (MCMC; Supporting methods) to simultaneously estimate model parameters and evaluate the alternative models based on their ability to explain the observations, i.e. relative growth rates, biomass nitrogen concentrations and fraction shoot biomass, for each experiment. In order to find the best way to model the key effect of N assimilation cost ( $r_A$ ) among the N forms we compared four model variants: (1) all N forms have the same assimilation cost, which is estimated in the model; (2) each N form has a different assimilation cost, which are all estimated in the model; (3) assimilation cost for each N form has fixed values as estimated by Zerihun (1998; mixed forms have average values between pure forms); and (4) assimilation cost for



**Figure 1.** Illustration of the effects of N form on plant growth and shoot:root ratio. Under balanced growth (Eqn 3) the relative C gain (dashed lines) must match the relative N uptake (solid lines), which corresponds to the intersections of the lines. Compared to growth on inorganic N (iN, dark blue lines, point a) growth on organic N (oN, light green) has two effects: (i) an increase in net C gain (point b) because of the lower N assimilation costs (the C bonus), and (ii) a lower relative N uptake because of lower N acquisition per root biomass, which in turn reduces the matching net C gain (point c). However, both effects (i) and (ii) contribute to reduced biomass fraction shoot, i.e. increased root:shoot ratio.

iN was taken from Zerihun (1998), but the costs for oN and ioN were estimated in the model.

The MCMC method results in a sample from the probability distribution of each parameter, from which the most likely value and confidence intervals are readily calculated. The MCMC output was also used to compare the likelihood of the alternative models based on deviance information criterion (DIC), telling us objectively which model is better while controlling for differences in the number of parameters and associated 'over-fitting' (Spiegelhalter *et al.* 1998).

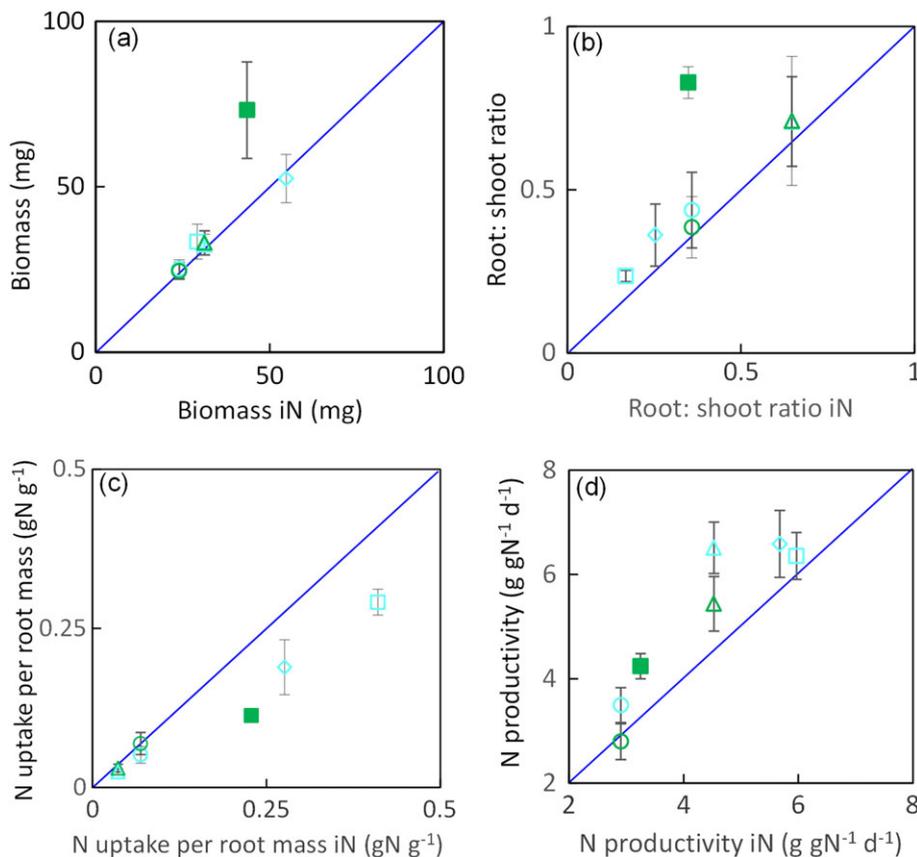
Based on the estimated parameters we evaluated the effect of variation in N and light availability to determine under which conditions N form is most important. In addition, to evaluate the total effect of the positive effect of the C bonus and the negative effect on root N acquisition rates for oN compared to iN, we modelled a scenario of variation in the relative availabilities of inorganic and organic N forms along a gradient in total N availability. Because we do not address soil processes we model N availability as seen by the root, i.e. N acquisition per root biomass, rather than soil N concentration in the soil *per se*. It has been observed that when total soil N was abundant, root acquisition of oN ( $u$ , measured on seedlings growing in

nutrient solution) is lower than that of iN, but both are similar when soil N was scarce (Warren 2009). Based on these observations we modelled the gradient of soil N availability by assuming that root N acquisition ( $u$ ) of oN relative to iN decreased from 100% to 0% as iN availability increased from 0 to high values.

## RESULTS

### Observed effects of N form on growth, allocation and biomass N concentration

Based on the data obtained from the experiments (Figs. 2 and S3), we calibrated the model and calculated the relevant variables for our analysis. We calculated relative growth rate from biomass, growing time, and initial biomass or seed weight, which we also used to estimate biomass at 20 days of growth (Fig. 2a). Root N acquisition is equal to plant N/root mass. In addition we calculated N productivity (the growth rate per plant N; Ågren & Bosatta 1998), as relative growth rate/biomass N concentration. The results showed rather similar growth rates among N forms, although in the experiment Arab 2 growth rate was higher for organic N (oN) than



**Figure 2.** Measured growth and N use in experiments with seedlings growing on different N forms. N treatments are: Inorganic N (iN, dark blue line), a mix of inorganic and organic N (ioN, cyan symbols), organic N (oN, green symbols). Error bars show 1 SD. Experiments (Table 1) are Arab 1 – open squares, Arab 2 – filled squares, Poplar – diamonds, Pine – triangles, Spruce – circles. Because growing time differed among experiments, biomass (a) was estimated for a common growing time of 20 days, based on the measured biomass at the end of each experiment and the initial plant (or seed) weight, assuming a constant relative growth rate. Within each experiment, a higher biomass corresponds to a higher relative growth rate. Nitrogen uptake per root biomass (c) is calculated as plant N content divided by root mass. Nitrogen productivity (d) is biomass growth rate divided by plant N content.

inorganic N (iN). In this experiment there was also a higher biomass C concentration in the oN treatment (40%) than in the iN treatment (36%). Compared to the effects on growth, there were larger effects of N form on root:shoot ratio, root N acquisition and N productivity. Root:shoot ratio and N productivity were generally higher, and root N acquisition lower, for oN and mixed organic and inorganic N forms (ioN) than for iN. These differences were particularly large for Arab. 2 (see also Fig. S3).

### Differences in the costs of N assimilation among N forms

Using the measured root N acquisition as input data we calibrated the model parameters to match the observations of relative growth rate, fraction shoot and biomass N concentration (see Model evaluation). Because the assimilation costs of different N forms was a main focus of our analysis, we tested four different ways to represent the N assimilation cost (see Model evaluation). The different model versions were evaluated based on their DIC (Supporting methods), which was equal to 328.1, 327.8, 330.5, 326.6 for models 1–4, respectively. Thus, the selected best model (the lowest DIC) was model 4, where N assimilation cost for iN was taken from Zerihun (1998) but the costs for oN and ioN were estimated in the model.

The model was able to reproduce well most of the observed differences among plants and N treatments in each experiment based on the independent variables, root N acquisition and N form. Averaged across experiments mean  $R^2$ s were equal to 0.82, 0.70 and 0.36 for fraction shoot biomass, biomass N concentration and relative growth rate, respectively (see also Fig. S4).

Whereas all other parameters (Table S2) were assumed independent of N form, the key outcome of the model for our

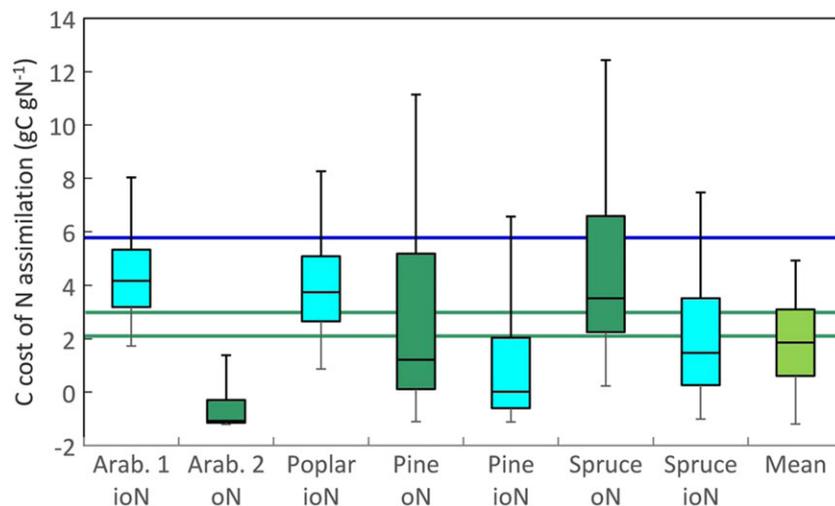
analysis is the assimilation costs of oN and ioN, for which the best estimates were lower than for iN in all experiments (Fig. 3). While results vary among experiments, the average of modelled N assimilation costs of oN and ioN across experiments was close to the theoretical values for assimilation of glutamine (Gln) and arginine (Arg) estimated by Zerihun (1998).

### Modelled interactions of light, total N availability and N form

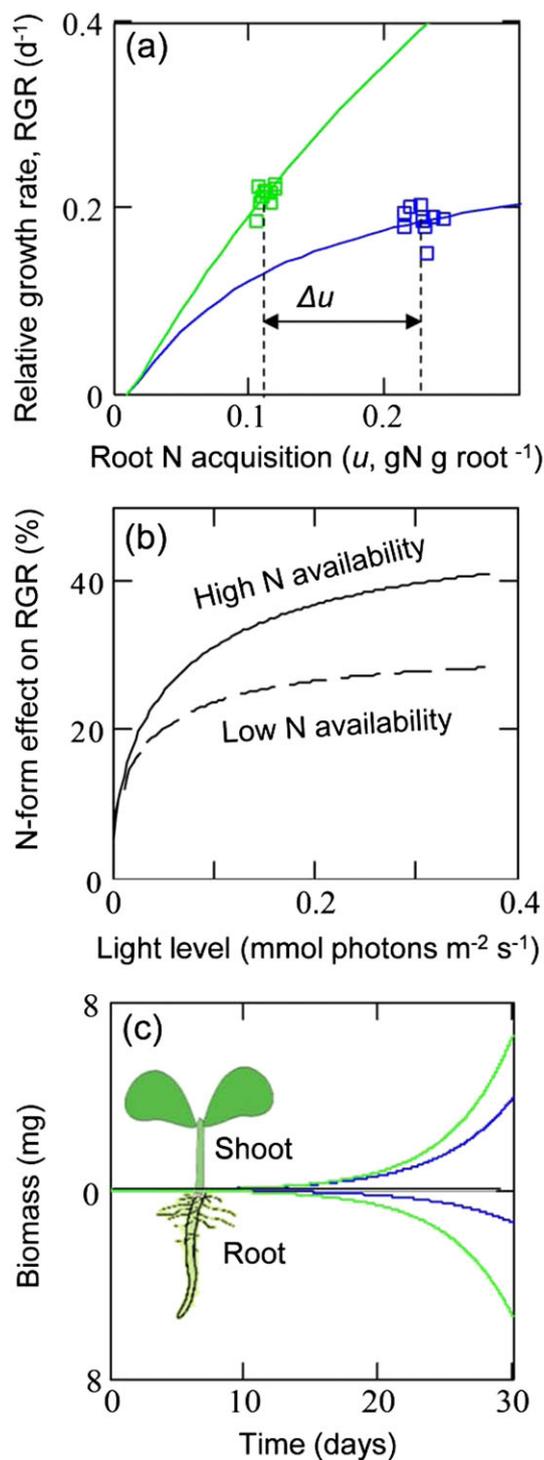
Our mechanistic model allows us to evaluate resource levels beyond those used in the experiments, and to determine under which conditions N form is most important. We found that the effect of N form is largest at high N and high light intensity (Fig. 4). In addition, we evaluated the effect of N form on growth under a soil scenario based on the observation that the relative availability of iN compared to oN increases along a gradient of increasing total soil N availability. Based on the parameters for Arabidopsis (Arab. 2 experiment) we found that growth rate and the nitrogen productivity were higher for oN than iN unless iN availability was at least three times higher than for oN (Fig. 5). Moreover, the root:shoot ratio was always higher for oN than iN.

### DISCUSSION

N form affects plant growth via its effects on root uptake and N assimilation cost. Using a tractable mechanistic model of plant growth we have shown that most of the effects of the form of N taken up by roots on plant growth, allocation and biomass N concentration can be explained by only two factors: N acquisition per root biomass and N assimilation costs. Importantly, we do not address how the availability of different N forms is controlled in the soil; rather, we focus on how a given acquisition of



**Figure 3. Model estimates of C costs of N assimilation.** Based on observed growth rates, N concentrations and shoot fractions, a Markov chain Monte Carlo method (Supporting methods) was used to estimate 75% lower and upper confidence intervals (bars), most likely value (ML, horizontal line in the bars) and 95% lower and upper confidence intervals (error bars) for each treatment, and for the mean among the ML values. Lines show theoretical values from Zerihun *et al.* (2008) for inorganic N ( $\text{NO}_3$ , dark blue line), organic N glutamine (Gln, lower green line) and organic N arginine (Arg, upper green line).



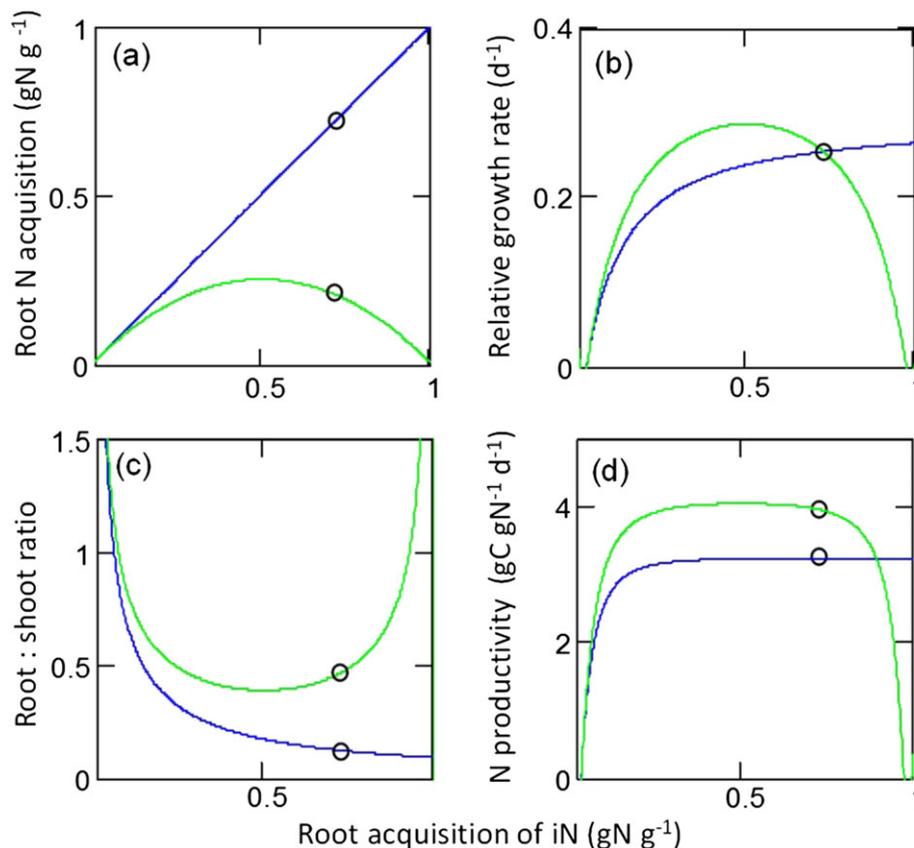
**Figure 4.** Modelled effects of N form, N availability and light on growth of Arabidopsis. (a): Measured (symbols) and modelled (lines) relative growth rate (RGR), where the vertical distance between growth on organic N (oN, light green line) and inorganic N (iN, dark blue line) is caused by the difference in N assimilation cost,  $r_A = 2$  ( $\text{gC gN}^{-1}$ ) for oN (Gln), and 5.8 for iN ( $\text{NO}_3$ ). The horizontal arrow ( $\Delta u$ ) shows the measured difference in root N acquisition ( $u$ ) where  $u = 0.13$  ( $\text{gN g root}^{-1}$ ) for oN and 0.23 for iN. (b) The modelled difference in RGR for oN compared to iN as a function of light level, shown for different N acquisition per root mass,  $u = 0.23$  (solid line) and  $u = 0.115$  (dashed line). (c) Because of their exponential growth, even relatively small difference in RGR (shown in a) between oN and iN leads to a large difference in biomass over time.

N per root biomass and the resulting N uptake affect plant growth. Growing seedlings on sterile agar plates in the Arabidopsis and poplar experiments ensured that the added N form was not converted to another form by microorganisms before it was taken up, and thus, true plant effects were observed. However, although non-sterile soil was used for pine and spruce, they responded to added N form similarly to the other plants, which indicates the experimental treatments were effective despite potential conversion of one N form to another by soil microorganisms. A key advantage of our approach was that we used total N uptake per root biomass as an independent (observed) variable, thereby controlling for differences in root N availability, which otherwise complicates the interpretation of N-form experiments.

While the model explains most of the observed differences among plants and N treatments, the slightly lower ability to explain effects on growth rate compared to the other plant traits is largely because of variation within each N treatment in the pine and spruce experiments (Fig. S4). However, the overall agreement between model and observations, as well as the consistently lower N assimilation costs for oN and ioN than iN (Fig. 3), strongly suggest that the model captures the key costs and benefits governing plant response to N form taken up. The effect of N form on N assimilation cost had previously not been quantified based on observations. Whereas bottom-up chemical calculations suggested negligible effects of N form on growth (Zerihun *et al.* 1998), experiments showed significant effects of N form, at times with higher growth and always higher root allocation for oN than iN (Fig. 2; Cambui *et al.* 2011). Here we partly reconcile these divergent findings by confirming the chemically estimated differences between N forms (Zerihun *et al.* 1998) by our independent estimates based on whole plant traits (Fig. 3), while at the same time showing that these differences do indeed affect plant growth and root:shoot allocation (Figs. 2, 4, S2).

In particular, the model shows that positive effects of oN relative to iN on growth can be ascribed to lower N assimilation costs (Fig. 3), which increases N productivity (Figs. 2, 5 and S2). However, because of a simultaneous (observed) negative effect on root N acquisition (Fig. 2c) only minimal effects on net growth were observed in most of the experiments (Figs. 2a, 4a). The net result of these counteracting effects on growth depends on their relative sizes and on the overall N availability, as discussed below. However, both these effects contribute to higher root biomass fraction for oN than iN treatments (Fig. 1), which explains the consistent observations of this effect (Fig. 2b, Cambui *et al.* 2011; Gruffman *et al.* 2012; Öhlund & Näsholm 2002).

The benefit of organic N is largest for small plants with high biomass N concentration under high light. While our experiments provide data for a limited range of light and N availability, our mechanistic model allows us to evaluate resource levels beyond those used in the experiments, and to determine under which conditions N form is most important. We find that the effect of N form is largest at high N and high light intensity (Fig. 4). The reason is that under these conditions biomass N concentration is high, and thus the cost of N assimilation for biomass growth is high and therefore influential relative to



**Figure 5.** Modelled growth of *Arabidopsis* on different N forms as a function of N availability. Solid lines show plant using inorganic N (iN, blue grey lines) and organic N (oN, light green lines) driven by a scenario of declining root acquisition of oN relative to iN according to (a). Despite this relative decline in oN acquisition, growth rate (b) remains higher for oN than iN until the relative acquisition per root mass of iN is three times higher than for oN (circles). At the same time, nitrogen productivity remains 20% higher (d) and root:shoot ratio three times higher for oN than iN (c).

other costs. Although this prediction appears to be in conflict with the general belief that oN would be most important for plants growing under low N availability (Paungfoo-Lonhienne *et al.* 2012), recent measurements of N availability at the scale of roots (rather than bulk soil) indicate that, excluding a period immediately after N fertilizer addition, plants take up more oN than iN even in a fertile agricultural site (Brackin *et al.* 2015).

The positive relationship between biomass N concentration and N assimilation costs means that the importance of N form declines as plants grow larger. As plants grow, self-shading reduces intercepted radiation per leaf, reducing the optimal N concentration of the leaves at the same time as the production of low N tissues such as stems increases in many plants. However, for small plants even a small difference in growth rate may be important, especially in a competitive context. Although the difference in relative growth rate between the N forms in Fig. 2 appear rather modest, because of the exponential growth of these small plants it quickly leads to large differences in size over time (Fig. 4c). In summary, while our estimates of the difference in N assimilation costs between N forms agree with bottom-up chemically based estimates (Fig. 3), our whole plant perspective reveals that these apparently small differences scale up to significant effects on growth and allocation over time and may be more important than previously thought.

### Organic N is cheaper to use but more expensive to get than inorganic N – but what is the total effect on growth?

Previous studies on N form preferences has been focused on the premise that plants are adapted, or acclimate, to preferentially take up the N form most available to them (Boczulak *et al.* 2014; Scott & Rothstein 2011), yet our findings imply that not only the availability of different N forms is important but also the cost of assimilation once the N has been taken up. Here we found that, compared to iN, oN is cheaper to assimilate but sometimes more expensive to take up (N uptake per root biomass is lower). A key question is how the benefit of a lower N assimilation adds up with the higher uptake cost, i.e. when is the net growth effect positive and therefore oN should lead to a higher plant growth than iN?

Whereas the difference in N assimilation cost should be largely independent of environmental conditions, the uptake difference depends on how external factors vary among soils, in particular with soil N availability. In low N soils, mainly organic N forms are available (Inselsbacher & Näsholm 2012), whereas in fertile high N soils, NO<sub>3</sub> or NH<sub>4</sub> dominate (Nordin *et al.* 2001; Paungfoo-Lonhienne *et al.* 2012). In line with the hypothesized correlation between availability and plant preference, when total soil N

was abundant, root acquisition of oN ( $u$ , measured on seedlings growing in nutrient solution) was lower than that of iN, but both rates were similar when soil N was scarce (Warren 2009). Based on these observations we modelled a gradient of increasing total soil N availability and decreasing soil oN: iN ratio (Fig. 5). Despite the declining relative availability of oN in this scenario, the growth rate advantage for oN compared to iN persists as N availability increases, even until acquisition of iN per root mass is three times higher than for oN (Figs. 5a, d). This demonstrates that the benefit of cheaper N assimilation (the C bonus) may make oN preferable to iN despite lower availability in the soil.

### Organic N uptake promotes NUE by enhanced root growth and N productivity

An important question for agriculture and forestry is how the form of N taken up by plants affects productivity and nitrogen use efficiency (NUE). There are many ways to define NUE (Lassaletta *et al.* 2014) and perhaps the most relevant from a practical perspective is the yield per N added. This NUE can be viewed as the combination of two factors: (1) the fraction of added N taken up by the plants, and (2) N productivity – the growth rate per plant N (Ågren & Bosatta 1998). For crop production, crop yield (e.g. grain) per biomass would be a third factor in the NUE equation, which however is mainly determined at a later stage of growth than the seedling phase considered here. Thus, our results are by no means directly applicable for quantitative predictions of agricultural yield, rather the indicate in which direction N form may change NUE and by which mechanism.

Although the first factor, the fraction N taken up, is largely controlled by soil processes, such as competition with microbes (Wilson *et al.* 2013), beyond the scope of our model it is also influenced by root growth. Our analysis shows that root biomass fraction is always higher if plants are growing on oN than on iN (Fig. 5), which was partly because of lower root N acquisition oN in our experiments (Figs. 1, 2 and 4). This means that a larger proportion of biomass production is allocated to roots rather than above ground crop yield. However, in all our experiments, except for poplar, there was higher relative growth rate for oN than iN treatments (Figs. 2 and S2). This advantage in relative growth rate may quickly compensate for the effect of a higher root:shoot ratio over time (Fig. 4c) so that total above-ground yield will be larger for oN than iN despite higher root:shoot ratio. Importantly, although it does not contribute to yield directly, the increase in root growth associated with oN leads to larger total N uptake capacity, which increases the fraction of added fertilizer N taken up and reduces N losses from the field and thereby increases NUE over time (Paungfoo-Lonhienne *et al.* 2012). Indeed, higher N retention for oN than iN has been observed in pine and spruce seedling nurseries (Öhlund & Näsholm 2002).

Our analysis shows that the second factor of NUE, the N productivity, is also higher for oN than iN because of lower N assimilation costs, unless oN availability is much lower than iN (<4% in Fig. 5). Interestingly, under rising atmospheric

CO<sub>2</sub> this advantage of oN over iN (if in the form of nitrate) may increase further as the photo-respiratory fuelling of nitrate assimilation declines because of reduced photo respiration (Bloom *et al.* 2014).

In summary, although the empirical evidence is yet limited to a few species grown in laboratories, our results show that plant uptake of oN has a previously underestimated physiological advantage over iN in terms of lower N assimilation costs. Because the effect is strongest in small plants with high N concentration, especially where light is not limiting, these results are particularly relevant for establishment of tree seedlings in tree nurseries and agricultural crops, suggesting that NUE could be increased by increasing availability of organic N relative to inorganic N. While the magnitude of such an NUE effect may be smaller in other settings, its basis in fundamental biochemistry suggest that the relationship between N form and NUE should be of universal relevance for plant growth.

### ACKNOWLEDGEMENTS

O.F. and T.N. were supported by The Kempe foundations, The Swedish University of Agricultural Sciences (TC4F and Bio4E) and the research councils: The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, The Swedish Research Council and The Swedish Governmental Agency for Innovation Systems and Knut and Alice Wallenberg foundation. S.P. and R.O. were supported in part by the US Department of Energy through the Office of Biological and Environmental Research Terrestrial Carbon Processes program (DE-SC0006967). OF acknowledges support from the European Research Council Synergy grant ERC-2013-SyG-610028 IMBALANCE-P. We thank Marta Gallart and Gabriel Katul for valuable scientific input.

### REFERENCES

- Ågren G.I. & Bosatta E. (1998) *Theoretical Ecosystem Ecology: Understanding Element Cycles*. Cambridge University Press, Cambridge.
- Ågren G.I. & Franklin O. (2003) Root:shoot ratios, optimization and nitrogen productivity. *Annals of Botany* **92**, 795–800.
- Andersen K.M. & Turner B.L. (2013) Preferences or plasticity in nitrogen acquisition by understorey palms in a tropical montane forest. *Journal of Ecology* **101**, 819–825.
- Arkoun M., Sarda X., Jannin L., Laigné P., Etienne P., Garcia-Mina J.-M., Yvin J.-C. & Ourry A. (2012) Hydroponics versus field lysimeter studies of urea, ammonium and nitrate uptake by oilseed rape (*Brassica napus* L.) *Journal of Experimental Botany* **63**, 5245–5258.
- Ashton I.W., Miller A.E., Bowman W.D. & Suding K.N. (2008) Nitrogen preferences and plant-soil feedbacks as influenced by neighbors in the alpine tundra. *Oecologia* **156**, 625–636.
- Bloom A.J., Burger M., Kimball B.A. & Pinter P.J. (2014) Nitrate assimilation is inhibited by elevated CO<sub>2</sub> in field-grown wheat. *Nature Climate Change* **4**, 477–480.
- Boczulak S.A., Hawkins B.J. & Roy R. (2014) Temperature effects on nitrogen form uptake by seedling roots of three contrasting conifers. *Tree Physiology* **34**, 513–523.
- Brackin R., Näsholm T., Robinson N., Guillou S., Vinall K., Lakshmanan P., Schmidt S. & Inselsbacher E. (2015) Nitrogen fluxes at the root-soil interface show a mismatch of nitrogen fertilizer supply and sugarcane root uptake capacity. *Scientific Reports* **5**, 15727.
- Cambui C.A., Svennerstam H., Gruffman L., Nordin A., Ganeteg U. & Näsholm T. (2011) Patterns of plant biomass partitioning depend on nitrogen source. *PLoS ONE* **6**.

- Chapin F.S. III, Moilanen L. & Kielland K. (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. *Nature* **361**, 150–153.
- Choudhury B.J. (2001) Implementing a nitrogen-based model for autotrophic respiration using satellite and field observations. *Tropical Ecology* **2**, 141–174.
- De Vries F.W.T.P., Brunsting A.H.M. & Van Laar H.H. (1974) Products, requirements and efficiency of biosynthesis a quantitative approach. *Journal of Theoretical Biology* **45**, 339–377.
- Dewar R.C., Franklin O., Mäkelä A., McMurtrie R.E. & Valentine H.T. (2009) Optimal function explains forest responses to global change. *BioScience* **59**, 127–139.
- Franklin O. (2007) Optimal nitrogen allocation controls tree responses to elevated CO<sub>2</sub>. *New Phytologist* **174**, 811–822.
- Franklin O., Johansson J., Dewar R.C., Dieckmann U., McMurtrie R.E., Brännström Å. & Dybzinski R. (2012) Modeling carbon allocation in trees: a search for principles. *Tree Physiology* **32**, 648–666.
- Geisseler D., Horwath W.R. & Doane T.A. (2009) Significance of organic nitrogen uptake from plant residues by soil microorganisms as affected by carbon and nitrogen availability. *Soil Biology and Biochemistry* **41**, 1281–1288.
- Gruffman L., Ishida T., Nordin A. & Näsholm T. (2012) Cultivation of Norway spruce and Scots pine on organic nitrogen improves seedling morphology and field performance. *Forest Ecology and Management* **276**, 118–124.
- Gruffman L., Palmroth S. & Näsholm T. (2013) Organic nitrogen uptake of Scots pine seedlings is independent of current carbohydrate supply. *Tree Physiology* **33**, 590–600.
- Harrison K.A., Bol R. & Bardgett R.D. (2007) Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology* **88**, 989–999.
- Harrison K.A., Bol R. & Bardgett R.D. (2008) Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? *Soil Biology and Biochemistry* **40**, 228–237.
- Hill P.W., Quilliam R.S., DeLuca T.H., Farrar J., Farrell M., Roberts P., ... Jones D.L. (2011) Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. *PLoS ONE* **6**.
- Holst J., Brackin R., Robinson N., Lakshmanan P. & Schmidt S. (2012) Soluble inorganic and organic nitrogen in two Australian soils under sugarcane cultivation. *Agriculture, Ecosystems & Environment* **155**, 16–26.
- Inselsbacher E. & Näsholm T. (2012) The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytologist* **195**, 329–334.
- Jämtgård S., Näsholm T. & Huss-Danell K. (2010) Nitrogen compounds in soil solutions of agricultural land. *Soil Biology and Biochemistry* **42**, 2325–2330.
- Jones D.L., Healey J.R., Willett V.B., Farrar J.F. & Hodge A. (2005) Dissolved organic nitrogen uptake by plants—an important N uptake pathway? *Soil Biology and Biochemistry* **37**, 413–423.
- Lambers H., Chapin F.S. & Pons T.L. (1998) *Plant Physiological Ecology*. Springer, New York.
- Lassaletta L., Billen G., Grizzetti B., Anglade J. & Garnier J. (2014) 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environmental Research Letters* **9**, 105011.
- McKane R.B., Johnson L.C., Shaver G.R., Nadelhoffer K.J., Rastetter E.B., Fry B., ... Murray G. (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* **415**, 68–71.
- Miller A.E. & Bowman W.D. (2002) Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: do species partition by nitrogen form? *Oecologia* **130**, 609–616.
- Miller A.E., Bowman W.D. & Suding K.N. (2007) Plant uptake of inorganic and organic nitrogen: neighbor identity matters. *Ecology* **88**, 1832–1840.
- Näsholm T., Kielland K. & Ganeteg U. (2009) Uptake of organic nitrogen by plants. *New Phytologist* **182**, 31–48.
- Nordin A., Högberg P. & Näsholm T. (2001) Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia* **129**, 125–132.
- Öhlund J. & Näsholm T. (2002) Low nitrogen losses with a new source of nitrogen for cultivation of conifer seedlings. *Environmental Science & Technology* **36**, 4854–4859.
- Owen A.G. & Jones D.L. (2001) Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. *Soil Biology and Biochemistry* **33**, 651–657.
- Paine C.E.T., Marthews T.R., Vogt D.R., Purves D., Rees M., Hector A. & Turnbull L.A. (2012) How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. *Methods in Ecology and Evolution* **3**, 245–256.
- Paungfoo-Lonhienne C., Lonhienne T.G.A., Rentsch D., Robinson N., Christie M., Webb R.I., ... Schmidt S. (2008) Plants can use protein as a nitrogen source without assistance from other organisms. *Proceedings of the National Academy of Sciences, USA* **105**, 4524–4529.
- Paungfoo-Lonhienne C., Visser J., Lonhienne T.G.A. & Schmidt S. (2012) Past, present and future of organic nutrients. *Plant and Soil* **359**, 1–18.
- Ryan M.G., Hubbard R.M., Pongracic S., Raison R.J. & McMurtrie R.E. (1996) Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. *Tree Physiology* **16**, 333–343.
- Schimel J.P. & Chapin S.F. III (1996) Tundra plant uptake of amino acid and NH<sub>4</sub><sup>+</sup> nitrogen in situ: plants compete well for amino acid N. *Ecology* **77**, 2142–2147.
- Schmidt S., Mason M., Sangtiew T. & Stewart G.R. (2003) Do cluster roots of *Hakea acitities* (Proteaceae) acquire complex organic nitrogen? *Plant and Soil* **248**, 157–165.
- Scott E.E. & Rothstein D.E. (2011) Amino acid uptake by temperate tree species characteristic of low- and high-fertility habitats. *Oecologia* **167**, 547–557.
- Spiegelhalter D.J., Best N.G., Carlin B.P. & Van der Linde A. (1998) Bayesian deviance, the effective number of parameters, and the comparison of arbitrarily complex models. Research Report, 98–009.
- Thornley J.H.M. (1998) Dynamic model of leaf photosynthesis with acclimation to light and nitrogen. *Annals of Botany* **81**, 421–430.
- Warren C.R. (2009) Does nitrogen concentration affect relative uptake rates of nitrate, ammonium, and glycine? *Journal of Plant Nutrition and Soil Science* **172**, 224–229.
- Wei L., Chen C. & Yu S. (2015) Uptake of organic nitrogen and preference for inorganic nitrogen by two Australian native Araucariaceae species. *Plant Ecology and Diversity* **8**, 259–264.
- Wilkinson A., Hill P.W., Vaieretti M.V., Farrar J.F., Jones D.L. & Bardgett R.D. (2015) Challenging the paradigm of nitrogen cycling: no evidence of in situ resource partitioning by coexisting plant species in grasslands of contrasting fertility. *Ecology and Evolution* **5**, 275–287.
- Wilson A.R., Nzokou P., Güney D. & Kulaç Ş. (2013) Growth response and nitrogen use physiology of Fraser fir (*Abies fraseri*), red pine (*Pinus resinosa*), and hybrid poplar under amino acid nutrition. *New Forests* **44**, 281–295.
- Zerihun A., McKenzie B.A. & Morton J.D. (1998) Photosynthate costs associated with the utilization of different nitrogen-forms: influence on the carbon balance of plants and shoot-root biomass partitioning. *New Phytologist* **138**, 1–11.

Received 24 November 2015; received in revised form 23 May 2016; accepted for publication 24 May 2016

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** Measurements indicating a constant biomass N concentration (a) and exponential growth (b) of *Arabidopsis* seedlings. Symbols show shoots (circles), roots (triangles) and total biomass (squares) for seedlings supplied with a mix of glutamine and nitrate (ioN, turquoise symbols) and only glutamine (oN, green symbols). The lines in (b) show fitted exponential functions corresponding to a constant relative growth rate ( $B = 3.90 e^{0.082t}$  for ioN and  $B = 3.50 e^{0.11t}$  for oN). Growing days ( $t$ ) is the number of days passed since the plants were transferred to the experiment. The growing conditions were equal to those in the experiment Arab 1 (Table 1).

**Figure S2. Optimal plant growth and the effects of N assimilation cost and root N availability.** Upper panels show how the lower assimilation costs of organic nitrogen uptake (dashed light-red lines) reduce total C costs relative to inorganic nitrogen uptake (solid light-red lines) leading to higher maximal net C gain defined as the difference between total C gain (dark green line) and costs (vertical arrows in c). It also slightly increases optimal N concentration, which, at fixed soil N availability, requires increased fraction root at the expense of shoot to support N uptake (b). Lower panels show how reduced root N acquisition because of reduced soil N availability (dashed versus solid lines in d–f) increases C costs to maintain a given plant N concentration (red lines in d) because more root

biomass is required and thus less shoot biomass fraction is allowed (e). The increased root costs and reduced shoot biomass fraction required to maintain a given N concentration reduces the optimal N concentration (f).

**Figure S3.** Growth, allocation and N concentration in *Arabidopsis* (Experiment Arab. 2). Modelled (lines) and measured (points) values are shown for growth on oN (Gln; green) and iN ( $\text{NO}_3$ ; dark blue). The horizontal (x-axis) separation of measured values shows the difference in root N acquisition (N uptake per root mass) between the N sources ( $\Delta u$  in a). The vertical difference between lines in panel a, c and d is the result of the difference in N assimilation costs between the N forms. The difference in the slopes of the lines in b ( $\Delta P_N$ ) reflects the difference in N productivity.

**Figure S4.** Modelled (y axis) versus measured (x axis) growth rate, biomass N concentration, and fraction shoot. N forms supplied were inorganic N (iN, dark blue symbols), a mix of inorganic and organic N (ioN, light cyan symbols), organic N (oN, green symbols). Symbol shape represents experiments (Table ), in upper panels: Squares – Arab.1, diamonds – Arab.2, crosses – Poplar, and in lower panels: Triangles – Pine, Circles – Spruce. The three groups of points in relative growth rate (RGR) for pine and spruce (panel d) correspond to low, medium and high N treatments from left to right, indicating that the correlation between modelled and measured values within each N level is low but high among N levels.

**Table S2.** Model parameters