# Organic nitrogen drives shifts in carbon allocation at multiple levels along the plant - soil continuum

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**Abstract**
Is nitrogen just nitrogen from a plant´s perspective or does the form of nitrogen, inorganic or organic, matter? In this paper we combine data from several experiments in which the form of nitrogen (N) supplied to plant, but not the amount, was varied. We present data from a breadth of systems spanning the model plant Arabidopsis where intrinsic responses in carbon (C) allocation patterns could be observed in the absence of microbial interactions on sterile agar to pot-grown conifer trees where a DNA metabarcoding approach was used to describe the root associated microbiome. Taken together, these studies point to a fundamental difference between organic and inorganic N as nutritional sources for plants and microbes. Our results provide evidence that organic N, in contrast to inorganic N, promotes growth of roots, root hairs and mycorrhizal fungi. Biochemical shifts in C contents of shoots and roots suggest a C-bonus from organic compared to inorganic N, possibly explained by a smaller fraction of C partitioned to N assimilation.

**Key words**
organic nitrogen, carbon allocation, soil microbiome, DNA metabarcoding, root development

## Introduction

Early studies illustrated that plants are well suited to take up and grow in a nutritionally replete condition on many different organic nitrogen forms (Hutchington and Miller 1911) in addition to the inorganic forms ammonium and nitrate. In the period that followed World War II this view narrowed as the importance of inorganic N was recognised as the driving force behind impressive productivity gains that were achieved during the green revolution. As a historical consequence then, this view more or less prevails when considering both natural ecosystems and agriculture. Beginning in early 1990s a series of seminal papers rejuvenated the scientific interest of plant organic nitrogen nutrition (Chapin et al. 1993; Näsholm et al. 1998; Paungfoo-Lonhienne et al. 2008). Following on from these studies, molecular, physiological and ecological studies have demonstrated the intrinsic capacity of plants to acquire and use simple organic nitrogen compounds. For instance, transporters mediating root uptake of amino acids have been identified in and functionally characterised using forward and reverse genetic approaches with the expression of these genes shown to correlate in the presence of amino acids in the root media (Hirner et al, 2006). Also, new methodology to study soil nitrogen fluxes using a minimal invasive *in situ* microdialysis technique has indicated that amino acids are potentially much more prevalent in soils in a range of different ecosystems than previously recognized (Inselsbacher and Näsholm 2012; Brackin et al. 2015). The extent to which organic N is used by plants is, however, inherently difficult to study and no method is currently available that enables us to quantify organic N nutrition of plants. Consequently, the debate on the potential quantitative role has lingered and consensus is yet to be reached in this matter.

The current contribution takes a different approach to the issue of plant organic N nutrition by focussing on the inherent biochemical differences between organic and inorganic nitrogen forms and the ramifications of these differences for plant performance, physiology and soil ecology. We report on differences in plant performance and biomass partitioning from studies of small Arabidopsis plants grown in sterile culture as well as from studies of conifer seedlings grown in pots. We then broad our discussion to consider the distinct differences in effects of organic and inorganic N on the soil microbiome with a focus on root-associated ectomychorrhiza. Based on these results we speculate that in addition to the quantities of N available to plants,

the composition of the soil N pool has a strong impact on plant growth and partitioning of biomass, which may in part be facilitated by a shift in the composition of soil and root associated microbiome.

**Results and Discussion**

*Biomass allocation*

To a degree, plant growth and development is plastic and responsive to the environment. In order to first examine the potential extent of the inherent plasticity of the plant in the absence of microbial interactions, sterile conditions were used to provide a simplified system where different N forms can be applied in known, stable and equivalent concentrations (for methods see Cambui et al. 2011). On sterile media Arabidopsis root biomass allocation is significantly higher both in relative and absolute terms in response to a sole N source of glutamine compared to an equivalent N level supplied as nitrate (Fig. 1). The differences in the gradient of the respective lines of best fit illustrate a fundamental developmental shift at a whole plant level is dependent on N-form.

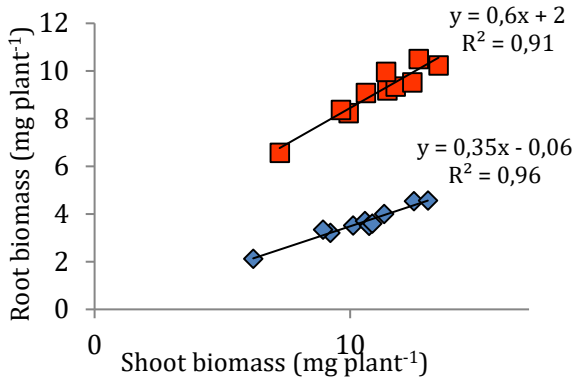


**Figure 1. Shoot and root biomass of small Arabidopsis plants grown on agar plates and supplied with either 3 mM nitrogen either in the form of nitrate (blue diamonds) or glutamine (red squares). Plants were grown for three weeks under standard conditions (see Cambui et al. 2011) and shoots and roots harvested.**

In the same Arabidopsis plants, C concentrations of shoots increased from 35 to 39 % and for roots from 38 to 40 % of dry weight. Although a crude indication of metabolic patterns of partitioning, these data suggest organic N has a significant impact on the C-status of plants. As glutamine contains carbon and is more highly reduced than nitrate, it is plausible the higher C-concentration in roots and shoots from plants grown with a glutamine N source is a consequence of a lower demand for C required in nitrate assimilation through to the GS-GOGAT cycle. Alternatively, N form-dependent influences on C-concentration may also represent a shift in C allocation between storage and growth. Interestingly, the most heavily down regulated genes in response to glutamine- relative to nitrate-grown plants observed in a microarray study coupled to this experiment are Rubisco small chain subunits, which points to a regulated decrease in allocation to photosynthetic machinery.

In addition to the N-form dependent shifts in physical and metabolic C allocation observed at the whole plant level, subtle but functionally important differences can also be observed within tissues. In most crops root development does not typically generate a direct economic return, however relatively small C investments below ground may indirectly amplify above ground yield. For instance, we observed root hair length in glutamine fed Arabidopsis on sterile plates to be stimulated 3-fold. While this represents a significant increase in root surface area for the amount of C allocated, in reality it is small compared to the potential efficiency gains in N and water uptake that can be leveraged by a plant's C investment in mutually beneficial relationship with ectomycorrhizal (ECM) fungi. ECM interactions (Fig. 2A & 3) and root development (Fig. 2B) were therefore considered in soil-grown trees. For this purpose we used a selection of *Pinus radiata* tree clones grown in pots and treated with equivalent N levels of either ammonium nitrate or L-arginine. An increase in lateral root branching and overall growth in response to organic N compared to inorganic N (Fig. 2B).

A N-form treatment effect was observed in the ECM colonisation rate (Fig. 2A). When classified by order, that is by the physical branching relationship to the root system architecture, the ECM colonisation rate was about 20% higher in L-arginine treated trees compared to ammonium nitrate

treated tree. As a rough quantitative measure, this suggests a greater C allocation to ECM is afforded under the conditions created by an L-arginine treatment.

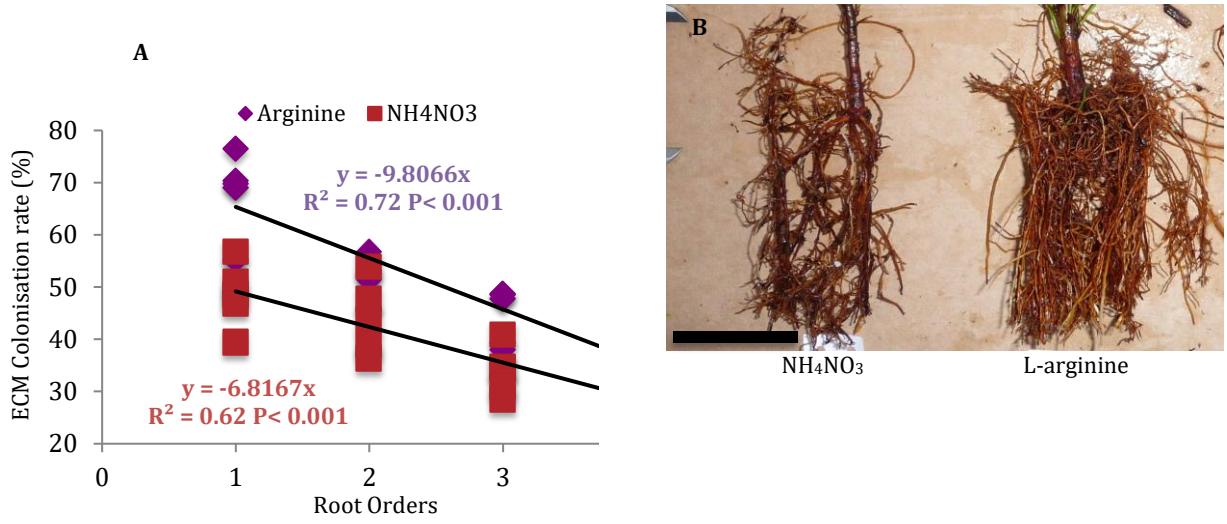


**Figure 2. ECM colonisation rates across five greenhouse-grown *Pinus radiata* genotypes in response to L-arginine (purple rombs) and $NH_4NO_3$ (red squares). Points represent the rate of colonisation resulting from the assessment of ca. 250 intersections by treatment and genotype for respective root orders (order 1 roots are most distal, order 2 roots are defined by the branching of an order 1 root and so on) (A). A photograph of *Pinus radiata* roots from cuttings grown in forest nursery containers treated with N-equivalent levels of N as either ammonium nitrate (left) or L-arginine (right). This was taken from an independent experiment to that used for the ECM and metabarcoding study described herein (Bar = 5 cm) (B).**

*Study of the soil rhizosphere communities*

Two *Pinus radiata* clones were identified for a comparative study of ECM communities from a screen of ten based on their different growth responses to inorganic or organic N. The clones selected displayed either an increase in total biomass in response to L-arginine versus ammonium nitrate (clone 1) or were neutral (clone 2). We then used high throughput next generation sequencing technologies to describe soil and root associated microbiome communities. We used the MiSeq® sequencing platform to generate metabarcoded DNA sequence representative of the fungal community present in soil and root associated samples taken from the two greenhouse-grown *Pinus radiata* clones treated with equivalent N levels of either $NH_4NO_3$ or L-arginine. Filtered and quality controlled sequence data was generated using the UPARSE pipeline (Edgar, 2013) that could be assessed for community richness and composition (Fig. 3).

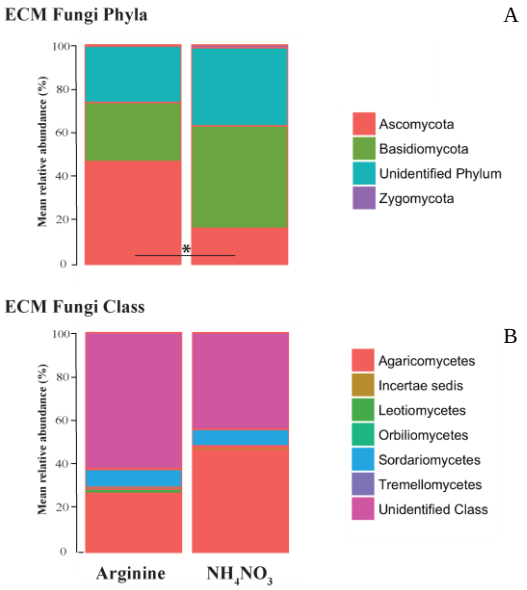


**Figure 3. Mean relative abundances of ECM-fungal phyla (A) and class (B) in response to the addition of the two N-forms. Mean was obtained from pooling relative abundances of two *P. radiata* genotypes (n = 6). The asterisk highlight significant phylogenetic differences across N treatments (*P < 0.05).**

The ECM community structure displayed a N-form treatment-induced difference, albeit only to the level of phyla owing to the current poverty of fungal sequence databases relative to actual biodiversity. Our biological interpretation of the metabarcoding data is thus cautioned by a distinction between observed versus functional richness and structure. As these measures are based on the conventional arbitrary sequence similarity cut off of 97 % to define operational taxonomic units (OTUs), functional richness and structure may indeed be influenced by a dominant role that may not be reflected by sequence abundance. Nonetheless it does appear that the host tree in this case, as the supplier of C to the plant-microbiome economy can influence the microbiome in manner that depends on the N environment.

## Conclusions

Nitrogen availability is known to exert a strong control over plant growth, biomass partitioning and soil microbiome and N fertilizers are key drivers of productivity in managed ecosystems. The current study shows that not only the N-amount, but also the N-form controls these features. The influence of N-form on whole plant C allocation patterns and the complex and dynamic interaction with the soil microbiome justifies greater effort in seeking a deeper understanding of organic N as a driver of natural variability in N-related productivity as well as N-management in agriculture and forestry. The greater partitioning of growth to roots, fine roots and mycorrhiza resulting from organic N is a candidate point of intervention to improve both nitrogen and water use efficiencies.

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