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Biology and Fertility of Soils
Cooperating Journal of International Society of Soil Science

ISSN 0178-2762

Biol Fertil Soils
DOI 10.1007/s00374-013-0818-2

Springer

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SHORT COMMUNICATION

Direct uptake and rapid decrease of organic nitrogen by *Wollemia nobilis*

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Received: 15 March 2013 / Revised: 20 April 2013 / Accepted: 9 May 2013

Abstract Organic nitrogen (N) can be directly taken up by many plants, particularly under low-temperature and N-limited conditions. The natural environment of *Wollemia nobilis*, shady conditions and shallow, acidic soils with high organic matter, led to the hypothesis that organic N might be a potential N source, although this species is living in a subtropical area. A pot experiment was carried out to investigate whether *W. nobilis* seedlings have the capability to take up intact organic N and whether the uptake of organic N contributes significantly to N acquisition for *W. nobilis*. Three ^{15}N-labeled N forms, ammonium (NH_4-N), nitrate (NO_3-N), or glycine, were injected into soils separately, and the tissues of plants were then harvested 6 and 48 h after injection. Our results demonstrated that *W. nobilis*, a subtropical species, has the capability to take up intact glycine as indicated by the enrichment of ^{13}C and ^{15}N in fine roots at a nearly 1:1 ratio. The uptake rate of glycine-N was faster than that of inorganic N, but which was only restricted in the short term (6 h). The absorbed glycine-N reduced quickly (in 48 h), indicating that organic N uptake did not contribute greatly to N acquisition for *W. nobilis*.

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Published online: 22 May 2013

Keywords Australian native plant species · Double labeling · Glycine · Subtropical · Wollemi pine

Introduction

Growing evidence of direct uptake of organic nitrogen (N) by plants has been obtained from studies on various plant community types (Näsholm et al. 2009). These ecosystems included mostly those under low-temperature conditions, such as arctic sedge, alpine tundra, and boreal forest (Chapin et al. 1993; Näsholm et al. 1998; Persson et al. 2006). However, studies on the species in tropical or subtropical communities are still limited, particularly in the southern hemisphere, although there have been several Australian species being studied (Schmidt and Stewart 1999; Warren 2006; Kahmen et al. 2009; Pfautsch et al. 2009; Warren 2009).

Wollemi pine (*Wollemia nobilis* W. G. Jones, K. D. Hill, and J. M. Allen), a newly discovered coniferous tree species in the Araucariaceae family (Jones et al. 1995), was once flourished in the Jurassic and Cretaceous periods (Hill 1997). It is only found naturally in the Blue Mountains approximately 150 km northwest of Sydney, Australia (Offord et al. 1999), where there is a warm region with high humidity (Benson and Allen 2007). How this ancient species survived is a mystery. It has been known that the soil is very shallow and sandstone-derived boulder alluvium with high organic matter content, but low nutrient levels (Offord et al. 1999; Benson and Allen 2007), plus extremely acidic pH in the range 3–4 (Benson and Allen 2007). Amino acids (simple form of organic N) are often presented in significant amounts in acidic organic soils (Taylor et al. 2004). Such soil may contain relative more organic N than fertile soils, which led to the hypothesis that dissolved organic N might be one alternative N source for *W. nobilis*, and the use of

multiple N sources might be one of the factors that enable this species to survive in nutrient poor soils.

The study aims to test (1) whether *W. nobilis* has the capability to take up organic N and (2), if it does, whether the organic N uptake contributes largely to N acquisition for this species. In this study, three forms of ^{15}N-labeled N (^{15}N-$(NH_4)_2SO_4$, ^{15}N-KNO_3, or ^{13}C and 2-^{13}C-^{15}N-glycine) were supplied to 3-year-old *W. nobilis* seedlings which were then harvested at 6 and 48 h after injection.

Materials and methods

Experimental design and sampling

The seedlings of *W. nobilis* were provided by the Toolara Nursery, Queensland, Australia. All stock plants were 3-year-old uniform cuttings without tap roots, grown in a 220-cm^3 plastic tube in a 50 % perlite/50 % pine bark peat (boiled and hammer-milled pine bark) potting mix. Vertical ribs inside pots prevented root coiling. The seedlings were fertilized with the 3.5 kg Osmocote (N/P/K, 11:4.8:14.9) and the 500 g MicroMix per m^3 of potting mix (pH 5.5). Thereafter, they were foliar sprayed with EX7 3 g L^{-1} (Grow Force Australia Ltd.; N/P/K, 20.8, 3.3, and 17.4 %, respectively). Seedlings were watered daily in the glasshouse under natural light conditions; the temperature ranged from 19.1 to 32.7 °C, and sun hours, from 0.0 to 13.5 h (mean 7.7 h), and relative humidity was 27–91 % (mean 64 %). Soil moisture was maintained at ca. 60 % of water holding capacity.

There were four treatments and two sampling times with four replicates in this experiment. The treatments included were as follows: CK (without any N application), glycine (^{13}C and ^{15}N labeled), $(^{15}NH_4)_2SO_4$, and ^{15}N-KNO_3. The 2-^{13}C,^{15}N-glycine used here has two advantages. Firstly, an equality of ^{13}C and ^{15}N addition to soil avoids the probability of detecting a significant ^{13}C uptake induced by universally labeled glycine (U-$^{13}C_2$,^{15}N-glycine) addition (Weigelt et al. 2005). Secondly, the non-carboxyl group (C-1 is the carboxyl group, and C-2 is the non-carboxyl group in the molecular structure of glycine) prevents a rapid reduction in ^{13}C/^{15}N ratios in plant materials which have been observed in both 1-^{13}C,^{15}N-glycine and U-$^{13}C_2$,^{15}N-glycine uptake experiments (Näsholm et al. 1998; Weigelt et al. 2005).

Glycine-2-^{13}C-^{15}N (99 at.% ^{13}C, 98 at.% ^{15}N; Sigma-Aldrich), $(^{15}NH_4)_2SO_4$ (10.65 at.% ^{15}N; Sigma-Aldrich), ^{15}N-KNO_3 (10.30 at.% ^{15}N; Sigma-Aldrich), or water (as the control) were injected separately into the soil. A total of 21 mL of treatment solution was injected with a stainless steel syringe needle (14 G) (Popper, New York) at three equally spaced points in the soil ca. 3–4 cm around each seedling (7 mL at each point) to provide a homogeneous distribution of the solutions in the pot soil. The final concentration of all supplied N forms was 10 μg N g^{-1} dry soils, which is falling in the range of soluble inorganic and organic N contents in the soil of natural *Araucaria* forests in Australia (Chen et al. 2002). To test the potential re-assimilation of the CO_2 derived from degradation of glycine by leaves, four pots of plants without any N fertilizers were placed next to the seedlings supplied with 2-^{13}C-^{15}N-glycine. The excess ^{13}C in plant materials in the glycine treatment was compared with that in the control plants.

All pots were arranged in a completely randomized manner. The incubation periods were decided based on the time that the ratios of ^{13}C/^{15}N in fine roots elapsed after injection of amino acids (McFarland et al. 2002). Seedlings were harvested 6 and 48 h (four replicates each time) after injection with N fertilizers. Leaves, stems, and fine and coarse roots were excised and collected separately for each seedling. The roots were thoroughly rinsed under distilled water to remove soil materials and then washed three times in 0.5 mM $CaCl_2$ solution (~15 min in total) to remove tracers from root surfaces. Finally, the excess $CaCl_2$ was washed off with distilled water. All plant samples were oven dried at 60 °C for 72 h and then ground to a fine powder for stable isotope analysis.

Calculation

To calculate mole ^{15}N excess and mole ^{13}C excess for each sample, data on N and C contents in plant tissue and atom percentage ^{15}N and atom percentage ^{13}C in excess of the natural abundances of ^{15}N and ^{13}C in the control were used. The quantities of N and C in plant tissues derived from the labeled glycine were calculated using the equations below as described by Näsholm et al. (1998) and by Taylor et al. (2004):

$$X_a = \left[\left(C_T[\%]\Big/12\right) \times \left(^{13}C_T \text{ atm}\% - ^{13}C_C \text{ atom}\%\right) \times f\right] \times 10^9 \quad (1)$$

$$X_a = \left[\left(N_T[\%]\Big/14\right) \times \left(^{15}N_T \text{ atm}\% - ^{15}N_C \text{ atom}\%\right) \times f\right] \times 10^9 \quad (2)$$

where X_a is the excess of ^{13}C or ^{15}N per dry weight of plant sample (in nanomole per gram per dw), C_T or N_T is the amount of C or N in the treatment plants with tracer application, C_C or N_C is the amount of C or N in the control plants without tracer application, and f is the enrichment factor of tracer (100/99 % for ^{13}C and 100/98 % for ^{15}N in this study). The equations above were also used to calculate the excess of ^{15}N in NH_4^+ and NO_3^- treatment samples. The enrichment factors are 100/10.65 % for NH_4^+ and 100/10.30 % for NO_3^-, respectively, in this study.

Data analysis

The excess ^{15}N or excess ^{13}C was compared using ANOVA with three fertilizers, two sampling times, and four plant parts as the fixed factors in this model. For all the significant results of ANOVA ($P<0.05$), the multiple comparisons were evaluated by using the LSD (for homogeneous data) or Tamhane (for nonhomogeneous data) method. The ratio of excess ^{13}C to excess ^{15}N in glycine treatment was compared with theoretical ratio (1:1) by using one-sample *t* test. Paired sample *t* test was used to compare the excess ^{13}C of those plants placed next to the glycine-treated plants and those of the controls. SPSS (Statistical Program for Social Sciences, version 16) was used to analyze the whole data set.

Results

The seedlings were growing well under the experimental condition as indicated by the concentrations of both C (54.5 ±2.4 %, SD) and N (0.9±0.1 %, SD) in leaves of *W. nobilis* which were similar to those in the other *Araucaria* species under healthy growth conditions (Franco et al. 2005). The relative high values of leaf carbon isotope discriminations (Δ) (mean −20.6±1.1‰) which was calculated from leaf $\delta^{13}C$ and negatively related with intrinsic water use efficiency (Farquhar et al. 1989) further reflected that *W. nobilis* seedlings were under well-watered conditions (Seibt et al. 2008).

Excess ^{15}N was detected in all plant tissues after injection with three fertilizer forms, i.e., 2-^{13}C-^{15}N-glycine, $(^{15}NH_4)_2SO_4$, and ^{15}N-KNO_3 (Fig. 1a, b, d). There was a greater amount of excess ^{15}N at 48 h compared with that at 6 h in the treatments injection with NO_3^--N and NH_4^+-N (Fig. 1a, b), while there was no significant difference in excess ^{15}N between 48 and 6 h in the glycine treatment (Fig. 1d). In the longer term (48 h), the NO_3^--N treatment had significantly greater excess ^{15}N in fine roots compared with the NH_4^+-N and glycine treatments, although there was no significant difference found between treatments in the short term (6 h; Fig. 1a, b, d). In the glycine treatment, excess ^{13}C was detected only in roots, but not in aboveground parts at 6 h, and there was excess ^{13}C detected in all tissues at 48 h (Fig. 1c). The ratio of excess $^{13}C/^{15}N$ in a fine root (0.73±0.05) was significantly differing from the theoretical ratio (1:1) at 6 h ($P<0.05$, $n=4$), while the ratio at 48 h (1.12±0.10) was not differing from 1:1 ($P=0.33$, $n=4$).

A shift of absorbed N from the root to the shoot was observed in all the treatments (Fig. 2). The absorbed N was

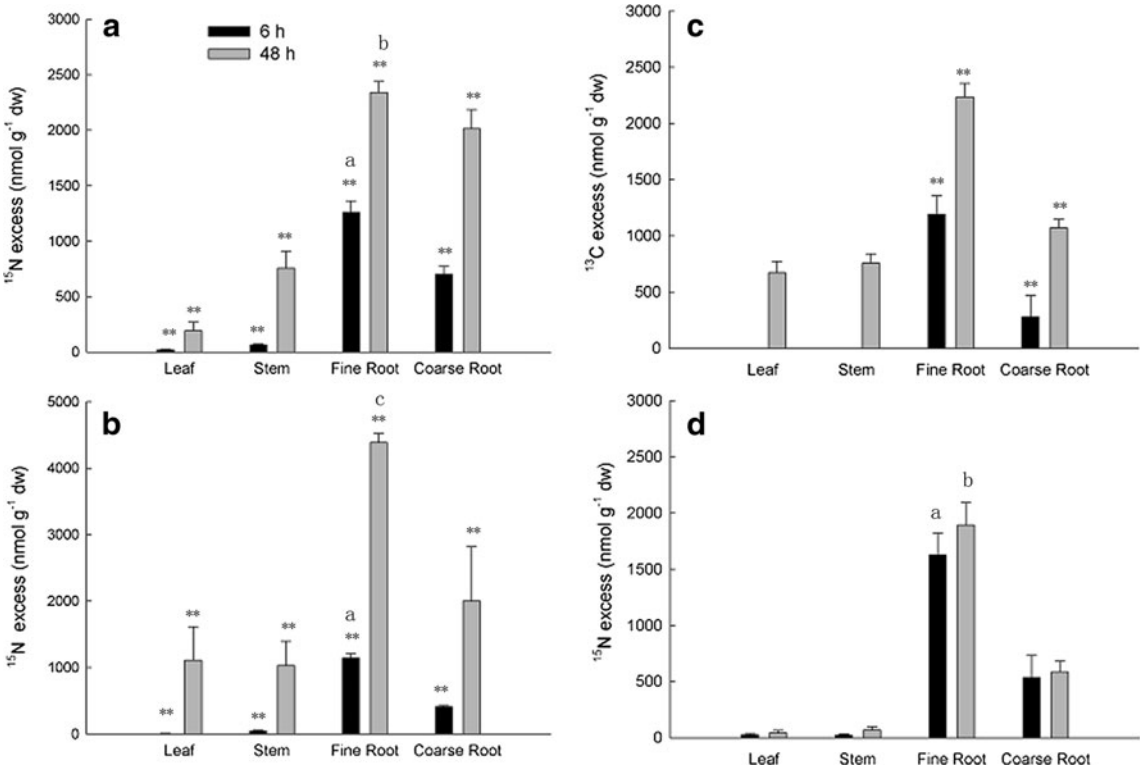

Fig. 1 Excess ^{15}N and ^{13}C in seedlings of *W. nobilis* after injection with labeled N fertilizers. **a** Excess ^{15}N in the NH_4^+-N treatment. **b** Excess ^{15}N in the NO_3^--N treatment. **c** Excess ^{13}C in the glycine treatment. **d** Excess ^{15}N in the glycine treatment. *Vertical bars*, 1 standard error (SE). *Asterisk* means a significant difference of isotope data between 6 and 48 h. Different *letters* mean a significant difference between treatments

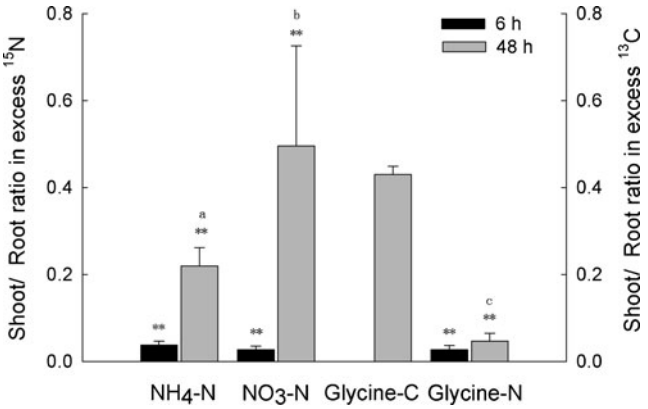

Fig. 2 Shoot/root ratios of excess ^{15}N or ^{13}C. *Vertical bars*, 1 SE. *Asterisk* means a significant difference of isotope data between 6 and 48 h. Different *letters* mean a significant difference between treatments

restricted in the root system at 6 h, while after 48 h, a significant fraction of the absorbed N was found in the aboveground parts. Among the three fertilizers, the largest fraction of absorbed NO_3^--N was transported to the shoot, followed by NH_4^+-N, and the transportation of glycine-derived N was least (Fig. 2). At 48 h after injection with glycine, there was a greater value of excess ^{13}C than excess ^{15}N in the whole seedling (Fig. 3a), and the potential loss of absorbed glycine-N was as much as ca. 30 μmol per seedling (Fig. 3b).

Discussion

Uptake of intact glycine

By using a pot experiment, we demonstrated that *W. nobilis* has the capability to take up intact glycine from soils, which is indicated by the ratio of excess ^{13}C/^{15}N being close to the theoretical ratio of 1:1 in the fine roots 48 h after injection with glycine. *W. nobilis* is living in a subtropical area, but the soil is N limited. Our study gives an example which demonstrated that even the plants living in warm environments could have the capability to take up organic N. Although previous studies indicate that the plants living in cold conditions tend to take up organic N (e.g., Persson et al. 2006), a few subtropical species have been reported to be able to take up intact organic acid as well, such as tropical savanna woodland (Schmidt and Stewart 1999). Additionally, the transporters that mediate the uptake of organic N have been identified both in mycorrhizal fungi and in plant roots (Näsholm et al. 2009), which inferred that uptake of organic N is likely to be a widespread adaptation strategy in natural ecosystems.

The higher values of total excess ^{15}N in the glycine treatment than those in the NH_4^+-N and NO_3^--N treatments at 6 h indicated that glycine uptake rate was faster than that of inorganic N in a relatively short time period. But after a longer time, NO_3^--N was likely to be preferred by *W. nobilis*. The preference for inorganic N over organic N is consistent with most previous studies (Harrison et al. 2007), but the uptake rate of NH_4^+-N is generally higher than that for NO_3^--N for most plants (Schmidt and Stewart 1999; Näsholm et al. 2009). The preference for NO_3^--N by *W. nobilis* is likely related to the acid soils the plant is living in, since NO_3^--N is the dominant N source in the acid soil (Cahn et al. 1992).

Rapid decrease of absorbed glycine-derived N

The total excess ^{13}C in whole plant being higher than excess ^{15}N 48 h after injection is in contrast with most previous studies (Schimel and Chapin 1996; Näsholm et al. 1998, 2001; Weigelt et al. 2005; Persson et al. 2006). However, similar results have also been observed in Norway spruce (*Picea abies*) (Nordin et al. 2001) and three grass species (Weigelt et al. 2003). The possible interpretation was that a part of the glycine-derived ^{15}N could be lost as an efflux to the soil (Nordin et al. 2001). Previous studies suggested that the absorbed glycine-N can be metabolized in fine roots via

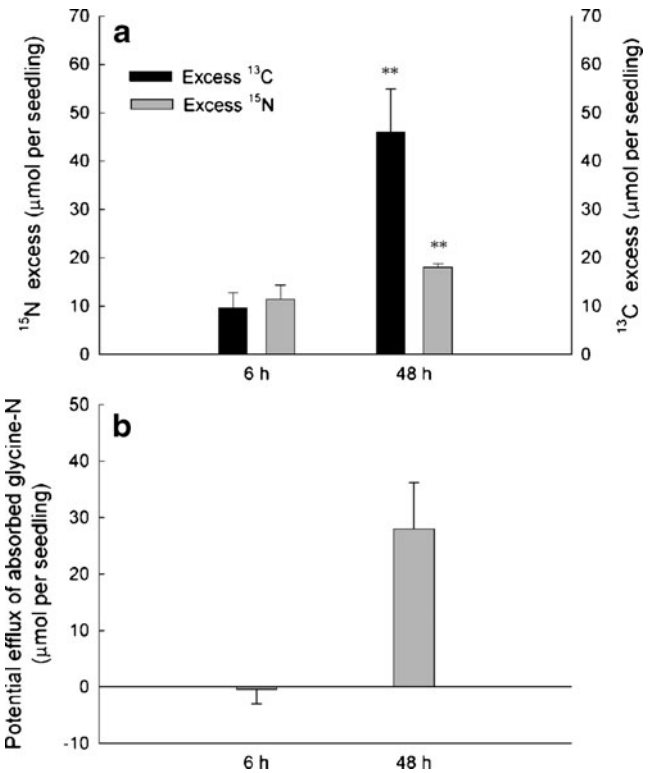

Fig. 3 Excess ^{13}C and ^{15}N in whole seedling of glycine treatment (**a**) and potential loss of absorbed glycine-N (**b**). *Vertical bars*, 1 SE. *Asterisk* means a significant difference of isotope data between ^{13}C and ^{15}N

transamination, possibly through the action of serine–glyoxylate aminotransferase, but not primarily via the serine hydroxymethyltransferase pathway which often occurs in leaves (Schmidt and Stewart 1999; Thornton 2001). This process resulted in the majority of absorbed Gly being transferred to L-Ser, followed by synthesis of L-Gln, L-Glu, and L-Ala. Based on the current study, it is hard to explain the results. Hypothetically, the amino acids were released as NH_3 from plants, or the ^{15}N in plant tissue has been diluted by other absorbed N. Maybe, there are some scarcely available nitrogen forms in the growth substrate, and it is possible that in 48 h, this plant can take up more N from the substrate than from the glycine which resulted in the dilution of the ^{15}N and not of the ^{13}C (Jones et al. 2005). Hence, it was not really a loss of glycine-derived N, but the uptake of alternative sources of N.

The rapid decrease of absorbed glycine-derived N observed in our study might constitute a potential mechanism for avoiding the accumulation of excessive NH_4^+, which would inhibit plant growth (Britto and Kronzucker 2002). This observation may also imply that the uptake of organic N probably plays a role other than N acquisition. A ^{14}C-labeled experiment revealed that up to 8 % of the total C in *Betula pendula* seedlings was due to amino acid assimilation, and it occurred particularly under deeply shaded conditions (Abuzinadah and Read 1989). To provide vigorous evidence, glycine metabolism in fine roots and translocation between plant parts in combination with influx and efflux of both amino acids and NH_4^+ would need to be studied further.

Conclusion

Our results clearly demonstrated that *W. nobilis* seedlings are able to directly take up a simple, soluble source of organic N, glycine, although inorganic N was the preference for the long term following injection. The interesting results we observed that a substantial of absorbed glycine-derived N decreased quickly. To explore the underlying mechanism, it is warranted to conduct in-depth studies on the glycine metabolism in fine roots and translocation between plant parts and the role of uptake of organic N.

Acknowledgments This research was supported by the Australian Research Council (FT0990547; DP0667184). The authors would like to thank the Toolara Nursery, Queensland, Australia, for providing *W. nobilis* seedlings; Ms. Marijke Heenan, Dr. Yumei Jiang, Dr. Fangfang Sun, and Dr. Xien Long for their laboratory assistance; and Prof. Gary Bacon for his logistic support.

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