Response of photosynthesis and nitrogen nutrition in *Juglans nigra* L. with different nitrogen fertilizers

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ABSTRACT

Black walnut (*Juglans nigra* L.) 1+0 half-sib seedlings were grown in a greenhouse for 4 months. The plants were fertilized with nitrogen as NH_4NO_3, $NaNO_3$, or $(NH4)_2SO_4$ at 0, 800, or 1600 mg N plant^{-1} applied exponentially. Root respiration, photosynthesis, chlorophyll *a* and *b* content, nitrogen and carbon levels, and growth parameters were measured. In general, plant dry weight was significantly higher for plants fertilized at 800 and 1600 mg than 0 mg with no difference between the 800 and 1600 levels. There were also some interactions between nitrogen source and level. Root respiration was not different between treatments. Photosynthesis and chlorophyll levels were higher with fertilization than without (with some interaction effects between source and level) and there was no significant difference between the 800 and 1600 levels. The levels of carbon and nitrogen, in the various plant components were more strongly affected by nitrogen source and level than any other parameters studied. These showed effects of both source and level of nitrogen as well as interaction. For the most part, plants that were fertilized had higher percentages of carbon and nitrogen than those that were not. In some cases, the 1600 mg N-fed plants had higher levels of these elements than the 800 mg N-fed plants and in some cases, there were no detectable differences. There were also interaction effects and effects due to nitrogen source, the latter being variable by plant organ. From these results, we conclude that there is not a strong nitrogen source preference for black walnut, but addition of nitrogen fertilizer is beneficial to plant growth and physiology.

Figure 1. Black walnut (*Juglans nigra* L.) seedlings used in the study.

INTRODUCTION

N fertilization has been shown to positively affect photosynthesis in most studies in the literature. This was shown in hybrid poplar (*Populus trichocarpa* Henry x *Populus balsamifera* (Dode) Farwell) (Ibrahim et al. 1998) and oak (*Quercus acuta* Thunb.) (Hikosaka and Hirose 2000). This relationship between N fertilization and photosynthesis is largely due to ribulose-1,5-biphosphate carboxylase (RUBISCO), which is required for photosynthesis and contains a large amount of N (Hikosaka and Hirose 2000). A study of *Betula papyrifera*, *B. lenta*, *Acer rubrum*, *Quercus rubra*, and some conifers showed that although photosynthesis increased with N addition. Photosynthetic nitrogen-use efficiency (PNUE), a measure of photosynthetic rate per unit leaf N, is higher with lower N. This suggests that at lower N additions, PNUE is more efficient (Fownes and Harrington 2004). This seems to mean that plants use N more efficiently at lower rates, but total photosynthesis increases.

There are relatively few studies in the literature that compared different N sources in relation to rate of photosynthesis. In a study of wheat (*Triticum aestivum* L.) and tomato (*Lycopersicum esculentum* Mill.), photosynthesis was maximized with nitrate (NO_3^-) over ammonium (NH_4^+) or a mixture (Lips et al. 1990). A study of photosynthesis in barley (*Hordeum vulgare* L.) showed the greatest rate of photosynthesis with a mixture of NO_3^- and NH_4^+ than with either as the sole N source (Lopes et al. 2004). This shows some of the variation in photosynthesis between species with different N sources. This would be due, in a large part, to how the plants reduce and transfer N. The differences in N assimilation and uptake would lead to different energy requirements: energy that has to be used for these processes would be diverted from processes involved in photosynthesis.

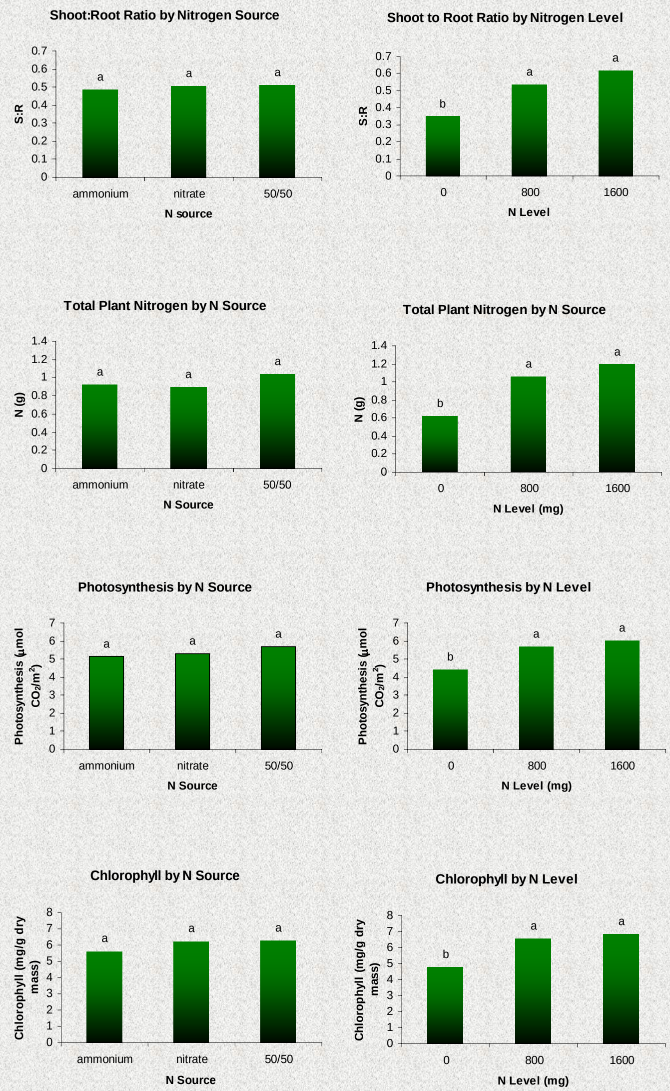

Figure 2: Response of *Juglans nigra* L. seedlings to fertilization with ammonium, nitrate, or half ammonium and half nitrate at 3 levels (0, 800, 1600 mg N per seedling) as measured in terms of shoot to root ratio, total N per plant, photosynthetic rate, and chlorophyll content. Different lower case letters signify significant differences in means.

METHODS

72 half-sib 1+0 black walnut (*Juglans nigra* L.) seedlings were grown at the Purdue Horticulture Plant Growth Facility. Seedlings were grown with Scotts Metromix 560® (Scotts Corporation, Marysville, OH, USA) in 10.65 l Tree-Pots™ (Steuwe and Sons, Corvallis, OR, USA). Fertilizer was applied as ammonium sulfate ($(NH_4)_2SO_4$), sodium nitrate ($NaNO_3$), or ammonium nitrate (NH_4NO_3) at 0, 800, or 1600 mg N per seedling. The fertilizer was added exponentially in 7 applications (one month following planting and every 2 weeks thereafter). These rates were determined from a previous study that showed significant physiological effects at 800 mg but not at 400 mg (Nicodemus et al. 2006). The design was a 3 × 3 factorial (N source by level) with 8 replications. Each seedling represents one replication

Photosynthesis and root respiration were measured using a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). For root respiration measurements, approximately 0.3 g fresh mass of new fine roots were placed in the measurement chamber and changes in $[CO_2]$ were recorded when the levels stabilized. For photosynthesis measurements, the levels of light, $[CO_2]$, and temperature were kept at standard levels representative of the ambient conditions. Photosynthesis measurements were taken for 2 cm² leaf area. The rate of $[CO_2]$ exchange was recorded when the rate stabilized.

Following photosynthesis and root respiration measurements, plants were separated into leaves, new stems, old stems, and roots and dried. Samples were ground with a Wiley mill to pass a 20 mesh sieve. These ground samples were used to determine C and N concentrations. C and N were determined by combustion using a LECO-2000 CNS analyzer (LECO Corporation, St. Joseph, MI, USA).

ANOVA was used to determine significant differences and significant means were separated using Tukey's test of significant differences ($\alpha = 0.05$). All statistical analyses were computed using SAS (SAS Institute Inc. 2001).

Figure 3: Equipment used in this study. On the left, a LI-6400 portable photosynthesis system. On the right, a LECO-2000 CNS analyzer

RESULTS AND DISCUSSION

We found that shoot to root ratio ($p < 0.0001$), total plant N (p , 0.0001), photosynthesis ($p = 0.0020$), and total chlorophyll ($p < 0.0001$) were significantly affected by N fertilization at 800 and 1600 mg N per plant as compared to the control. There were no significant differences between sources (Figure 2) (S:R, $p = 0.9075$; total plant N, $p = 0.2002$; photosynthesis, $p = 0.5317$; chlorophyll, $p = 0.1080$). The increased shoot to root ratio with fertilization demonstrates that more of the plant C is being devoted to aboveground growth as opposed to belowground. Plants tend to grow more roots when nutrients are in low supply; therefore, the energy and C devoted to root growth is diverted from the aboveground parts. Also, at low levels of N, less N is available for chlorophyll formation, which would also tend to be reflected in a decrease in photosynthetic rate as found in this study. Because less leaves are grown, reduced whole-plants photosynthesis is expected and total dry mass decreases.

We would also expect to find an increase in root respiration with increasing N addition, because the rates of N-assimilating enzymes would increase. We did not find significant differences in root respiration in this study (data not shown), which could mean that the N was already assimilated and so enzyme activity had decreased.

REFERENCES

Fownes, J.H. and Harrington, R.A. 2004. For. Ecol. Manage. 203:297-310. **Hikosaka, K. and Hirose, T.** 2000. Tree Physiol. 20:1249-1254. **Ibrahim, L. et al.** 1998. Tree Physiol. 18:481-487. **Lips, S.H. et al.** 1990. J. Plant Nutr. 13:1271-1289. **Lopes, M.S. et al.** 2004. Funct. Plant Biol. 31:995-1003. **Nicodemus, M.A. et al.** 2006. *In* Proceedings of the 15th Central Hardwood Forest Conference, in press. **SAS Institute Inc.** 2001. SAS/START user's guide. Volume 82. Cary, NY, USA