NITROGEN FIXING TREES INFLUENCE CONCENTRATIONS OF AMMONIUM AND AMINO SUGAR-NITROGEN IN SOILS

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ABSTRACT

The recently developed Illinois Soil Nitrogen Test (ISNT) predicts corn's response to nitrogen fertilization. ISNT measures the combined concentration of ammonium and amino sugar-N in soils providing an alternative approach for N fertility assessment. This value corresponds with the labile soil organic N fraction. Actinorhizal (*Frankia*-nodulated, nitrogen-fixing) autumn olive trees (*Elaeagnus umbellata* Thunb.) increased ammonium plus amino sugar-N concentrations from 54 to 104 $mg\cdot kg^{-1}$ (146-280 lbs per acre in the top foot of soil) and actinorhizal European alder trees (*Alnus glutinosa* L. Gaertn.) from 54 to 98 $mg\cdot kg^{-1}$ (146-265 lbs per acre) on mine spoils. We found a significant, negative correlation (r= -0.681) between ammonium plus amino sugar-N concentrations and total N, suggesting that enriched mine spoils had increased ammonification and subsequent loss of N through nitrification and leaching. *A. glutinosa* increased soil ammonium plus amino sugar-N by 13%, decreased pH, and stimulated cation leaching of a fertile Mollisol at the University of Illinois Arboretum. At another location with similar Mollisols, *A. glutinosa* trees and the tree legume *Robinia pseodoacacia* L. had been planted as hardwood-plantation nurse trees and subsequently eliminated by competition. A history of interplanting did not result in soil ammonium and amino sugar-N values significantly greater than those in an adjacent hardwood plantation without N-fixing trees. Ammonium plus amino sugar-N concentrations of 314-391 $mg\cdot kg^{-1}$ (848-1,056 lbs per acre) in the upper 30 cm (one foot) of the Mollisols were not correlated with total N measurements and were greater than the N fertilization nonresponse value (>235 $mg\cdot kg^{-1}$ or 635 lbs per acre) at which corn becomes non-responsive to N fertilization. Results suggest that the ISNT is sensitive to increases in soil N fertility by N-fixing trees and has potential as a site-specific index of their soil improvement capacities.

Keywords: actinorhizal plants, *Alnus*, amino sugar-N, *Elaeagnus*, nitrogen fertility, nitrogen fixing trees

BACKGROUND AND INTRODUCTION

Nitrogen-fixing trees are major components of agroforestry systems. They can themselves provide wood, fiber, shade, shelter, fruit, animal fodder, and increased soil moisture through condensation of mist on leaves. At the same time they enrich the soil with nitrogen fixed by root-nodule-forming bacterial symbionts. The fixed nitrogen (N) is released to the soil by decomposition of N-rich tree litter and sloughed roots. The process is similar to that of the common, herbaceous agricultural legumes, and is carried out mainly by tree legumes capable of symbiotic N-fixation and the nonleguminous, nitrogen-fixing trees and shrubs nodulated by the actinomycete *Frankia*. Common, N-fixing tree legumes used in agroforestry include *Acacia*, *Prosopis*, *Robinia*, *Leucaena*, and many others. Not all tree legumes fix nitrogen. For example, leguminous tree species of eastern North America in the *Gleditsia* (honeylocust), *Gymnocladus* (Kentucky coffee tree), *Cercis* (redbud) and *Cladrastis* (yellow wood) genera do not symbiotically fix nitrogen. Among the 25 genera of actinorhizal plants with species used in agroforestry are *Alnus* (alders), *Elaeagnus* (autumn olive and Russian olive), *Casuarina* (Australian she oaks), *Myrica*, and *Gymnostorna*. By virtue of their abilities to colonize and tolerate raw, infertile soils and to survive harsh conditions, many N-fixing tree species are weedy, particularly on sites altered, disturbed or degraded by human activity.

Nitrogen-fixing trees increase total soil N, soil N mineralization, and N nutrient cycling (Bormann et al. 1993). Previous work has shown that total soil N accumulation around *Alnus glutinosa* L. Gaertn., mixed with hybrid *Populus*, decreased with distance from actinorhizal *A. glutinosa* stems and with soil depth at 4 cm increments (Dawson et al. 1983). In a related study, hybrid *Populus* height growth was positively correlated with the number of neighboring *Alnus* trees (Hansen and Dawson 1982). Soils of a mixed *A. glutinosa* and poplar plantation in Canada had increased total N and N availability at the early interplant stage (Cote and Camire 1985). *A. glutinosa* interplanting also increased the N content of leaf tissue and stems and branches of associated poplar trees.

Plantings with nitrogen-fixing trees can profoundly influence soil properties such as organic matter, N, P, nutrient cycling, and resultant growth of associated trees (Dawson et al. 1983; Friedrich and Dawson 1984; Paschke et al. 1989; Giardina et al. 1995; Hart et al. 1997; Rothe et al. 2002). The greatest growth of *Juglans nigra* L. in a set of interplantings with nitrogen-fixing *Alnus* and *Elaeagnus* trees occurred in an *Elaeagnus* interplanting with the highest net annual N mineralization rates (Paschke et al. 1989). The total soil N concentration (Kjeldahl) was not correlated with N mineralization rates nor *J. nigra* growth.

Rothe et al. (2002) used ion-exchange resin methods to measure total mineralized N in soil. The result showed that *Alnus rubra* Bong. interplanted with *Pseudotsuga menziesii* (Mirbel) Franco contributed to greater mineral N accretion to a 45-cm depth compared with pure *P. menziesii* plantings. Previous work on soil N pools of mixed *A. rubra* and *P. menziesii* stands revealed that soil N mineralization and nitrification increased with interplanting (Binkley et al. 1992; Hart et al. 1997). In stands of *A. rubra* interplanted with *P. menziesii*, resin available mineral NO_3^--N was 45% of total N compared with 66% in stands without *A. rubra* interplanting. This suggested that increased NO_3^--N leaching might have been an effect of *A. rubra* on soil N dynamics in a mixed interplanting (Van Miegroet and Cole 1984; Binkley et al. 1992, 1994).

A study by Cole et al. (1990) indicated that biomass, available nutrients and productivity of a second rotation *A. rubra* plantation declined after five years. A decrease in soil nutrients to support their own growth is one of several possible effects of nitrogen-fixing tree species hypothesized in a review paper by Binkley and Giardina (1998). The ratio of lignin to N in aboveground litterfall can also be used to predict net N mineralization rates in soils (Scott and Binkley 1997).

The acetylene-reduction assay for nitrogenase activity has been employed to estimate *in situ* nitrogen fixation rates, but fails to estimate soil N fertility adequately (Sylvia et al. 1999). Biological and chemical indices of potentially available soil N can be closely related to total soil N and total soil organic matter (Keeney, 1982). Most soils contain between 0.08% and 0.4% total soil N primarily in organic forms (Keeney, 1982). Indices of soil N availability, including total soil N and the estimates of soil NO_3-N, NH_4-N have not provided an acceptable basis for N fertilizer recommendations.

Even where total organic N and N mineralization rates are closely correlated, total soil N is not recommended as a sole index for soil capacity to supply N because it oversimplifies N-cycling and transformations in soil (Wang et al. 2001). Moreover, Walley et al. (2002) evaluated several soil available N indices to examine the size of the available N pool for crop uptake and found that most indices did not account for N accretion in crop production. The evaluation suggested that no single index is adequate to predict fertilizer N requirements as did the evaluation of Bundy and Meisinger (1994).

Estimates of net or gross mineralization can be determined by several methods including ^{15}N natural abundance, ^{15}N tracer, ^{15}N isotope-dilution, resin cores, buried bags, closed-top tubes, and aerobic incubations (Hart et al. 1994). Wang et al. (2001) argued that net N mineralization does not really represent N mineralization ability in soils since mineral N uptake by plants occurs simultaneously during the course of mineralization. Hence, they used the gross amount of mineralization as an index for N mineralization ability in soils. Numerous studies have emphasized the need to measure soil N availability indices in order to predict and evaluate available soil N reliably in crop production and N fertilization recommendations (Bundy and Meisinger 1994; Walley et al. 2002).

Soil scientists have attempted for some time to develop a rapid method to estimate potentially available soil N (Picone et al. 2002). Using biological incubation for assessment of N availability is time-consuming and not appropriate for routine available-soil-N testing because it involves soil incubation in the lab for estimating N mineralization in the field. Chemical methods of estimating potentially available soil N are appealing since they could provide a rapid and simple technique for assessment of N fertilizer recommendations. Unfortunately, they are also unlikely to account for field-measured N availability because they do not represent the exact plant-soil environment for microorganisms.

Research on soil nitrate measurements to predict crop N demand would likely provide the best estimates of potentially available soil N in the field according to Hong et al. (1990) and Magdoff et al. (1984). There have been several studies employing the hot KCl chemical extraction method for assessment of soil capacity to supply N (Smith and Li 1993; Jalil et al. 1996; Beauchamp et al. 2004) as an alternative to soil nitrate tests. Beauchamp et al. (2004) found that using hot KCl-extractable NH_4-N to predict N fertilization needs of *Zea mays* L. can obtain better results than other soil N assays.

Microdiffusion is widely employed to determine exchangeable NH_4^+, NO_3^-, and NO_2^- in soil extracts (Mulvaney 1996). Microdiffusion is relevant to microbial transformation processes of nitrification, denitrification, mineralization and immobilization. But inorganic forms of N are subject to rapid changes resulting in high temporal and spatial variability.

The Illinois Soil Nitrogen Test (ISNT) that we employ was described by Khan et al. (2001) and is a novel and potentially revolutionary development in soil N-fertility assessment. It may provide an accurate and simple index of soil N mineralization ability. The ISNT determines the combined concentration of inorganic ammonium nitrogen and the soil amino sugar-N fraction. Inorganic ammonium in agricultural soils of this study typically comprises 2-5% of total soil N. Soil amino sugar-N is a labile organic fraction comprising 5-10% of total soil N (Stevenson and Cole 1999) or 5-6% of total N in humic substances (Schulten and Schnitzer 1998). Amino sugar-N in soils originates from microbial activity rather than from higher plants (Parsons 1981). Therefore, the concentration of amino sugar-N in soils reflects the level of mineralizable N and soil capacity to supply N to promote plant growth.

A quantitative determination of individual amino sugar-N can be accomplished by using a steam distillation method for soil hydrolysates (Stevenson 1996). This procedure is time-consuming and also requires experience and skill to successfully implement. Using acid hydrolysis to predict crop yield under cultivation does not differentiate the distribution of amino sugar-N concentration in soils as a result of crop cultivation, and it is of limited value as an estimate of soil available N with respect to soil organic N distribution (Stevenson and Cole 1999).

A positive correlation between soil amino sugar-N plus NH_4-N values and check-plot yield was described by Mulvaney et al. (2004), using the ISNT. This simple and inexpensive method to determine soil N availability is sensitive and provides an estimation of soil capacity to supply plant-available N, which is related to the labile organic N-fraction. Monitoring of ammonium and amino sugar-N spatial patterns around nitrogen-fixing trees may provide a useful estimate of soil N fertility and its spatial and temporal variability.

The objectives of this study are: (1) to determine the influence of actinorhizal trees on soil ammonium plus amino sugar-N (hereafter referred to as amino sugar-N) patterns as a potential index of N mineralization, (2) to determine how plantings of different actinorhizal species in various soil types have altered soil concentrations of amino sugar-N, and (3) to describe amino sugar-N distribution with respect to soil depths in plots with and without N_2-fixing trees.

MATERIALS AND METHODS

Study sites

Three sites were employed in this study. The University of Illinois Arboretum is located on the east side of the University of Illinois campus. Arboretum soils are in the Flanagan and Drummer soil series: fine, montmorillonitic, mesic Aquic Argiudolls and fine-silty, mixed, mesic Typic Haplaquolls, respectively. Soils are dark gray and firm silt loam to silty clay loams formed in loess over glacial till (Mount 1982). A cluster of three *A. glutinosa* trees approximately 40 years in age was selected for soil sampling to determine amino sugar patterns under and outside the tree canopies. Bulk density of soils was 1.16 g/cm^3. A *Tilia americana* L. tree of similar age 20 meters (67 feet) away from *A. glutinosa* cluster was selected as a control. The sampled area is approximately 0.1 hectare (0.25 acres).

The Douglas-Hart Nature Center (DHNC), located at the western edge of Charleston, IL, was selected as a second site. It is approximately 25 hectares (62 acres) in area. The soil types are Raub and Drummer, which are fine-silty, mixed, mesic Aquic Argiudolls and fine-silty, mixed, mesic Typic Haplaquolls, respectively, formed in loess over glacial till (Hamilton 1993). The surface soils of this site are dark gray, silt loam to silty clay loam and somewhat poorly drained to poorly drained. The eastern half of the study site had native trees interplanted in a ratio of 2:1 with N_2-fixing *A. glutinosa* or *Robinia pseudoacacia* L. The other half had the same spacing of 3 × 3m (10 × 10 feet) but was not interplanted with nitrogen fixing trees. The plantation is 50 years old. *A. glutinosa* and *R. pseudoacacia* trees currently comprise less than 5% of tree basal area at 1.4 m (4.5 feet) in height in the interplanted half, having been eliminated by competition from interplanted and volunteer trees. The interplanted half had large dominant trees with 100% canopy cover, while the noninterplanted half had smaller trees and many canopy openings. The average soil bulk density at DHNC was 1.01 g/cm^3.

The third site, located adjacent to Kickapoo State Park in Vermilion County, IL, is on 45-year-old mine spoils, with mixed yellow-brown coarse sand, loam, and rock comprising the overburden of limestone, sandstone, and shale in the variable textured upper part of the soil profile. *Elaeagnus umbellata* Thunb., *A. glutinosa*, and control plots with *Salix interior* Rowlee or grass were sampled. The plots were in close proximity to one another in an area of more than 200 hectares (494 acres) of mine spoils. A thin *A. glutinosa* stand of 0.03 hectares (0.07 acres) with remnant *A. glutinosa* trees spaced approximately every 10 m (33 feet) and 60% canopy cover, a dense stand with complete *E. umbellata* canopy coverage of 0.03 hectares (0.07 acres), and adjacent control areas of 0.01 hectare (0.025 acres) with *S. interior* and grasses were sampled. The average bulk density of the mine spoils was 1.00 g/cm^3.

Soil sampling

At the University Arboretum, 18 points was randomly selected under the canopies of a cluster of three *A. glutinosa* trees, all approximately 50 cm (20 inches) dbh (diameter of tree stem at breast height = 1.4 m above the soil level), for soil sampling. Twenty-five points were randomly selected beyond *A. glutinosa* canopies within one crown radius distance beyond the edge of the leaf canopy (7m or 23 feet). Four randomly selected points were also sampled under the canopy of a nearby *T. americana* tree. Three randomly selected points were taken away from the canopy within one crown radius distance beyond the edge of the *T. americana* leaf canopy (6m or 20 feet). At the DHNC site sixteen randomly selected points were sampled within the area of tree interplanting with nitrogen-fixing nurse trees and six points within the area planted without nitrogen-fixing *A. glutinosa* and *R. pseudoacacia*. At Kickapoo State Park, there were four sample points randomly selected within *E. umbellata* stand, 18 points randomly selected within *A. glutinosa* stand, and three points randomly selected in nearby stands of grass and *S. interior* trees. Sample sizes were determined by available time for extraction and were proportional to areas in the different stands unobstructed by ponds, mulch or structures.

At each point, a 30 cm (12 inches) soil probe with 2-cm-internal (0.8 inches) diameter was used to collect soil samples in 10-cm increments (4 inches) (0-10, 10-20, and 20-30 cm or 0-4, 4-8, 8-12 inches) to a depth of 30 cm and an adjacent, 2-cm horizontally distant, 0-30-cm soil core. The samples were collected during June and July of 2004 and were dried the same day as sampled for 24 hours at 40^{o}C. Each soil sample was then ground to pass through a 2mm (0.08 inches) sieve.

Illinois Soil Nitrogen Test

The samples were analyzed using the ISNT technique described by Khan et al. (2001). This soil N test employs alkaline decomposition by heating with a strong base. Since the liberation of amino sugar-N as gaseous NH_3 is sensitive to heating, it was crucial to precisely control the temperature within a range of $48\text{-}49^{o}$C to obtain a reliable estimate of ammonium plus amino sugar-N. One gram (0.04 ounces) of ground, air-dried soil was treated in the Mason jar with 10 ml of 2 *M* NaOH. A 5-cm-diameter petri dish containing 5 ml of H_3BO_3-indicator solution was suspended from the lid of 1-pint Mason jar. The sealed jars were heated for five hours at $48\text{-}49^{o}$C. After five hours of ammonia diffusion, the H_3BO_3-indicator solution was titrated with 0.01 *M* H_2SO_4 to determine quantity of ammonium from amino sugar and ammonium N in the soil samples. For each subsample, triplicates were analyzed to insure consistency. One Mason jar containing an artificial soil with a known amino sugar-N concentration was used in each incubation analysis to ascertain the accuracy of the results.

Additional soil chemical analyses

A random subsample of three of the 0 to 30-cm cores from each treatment was selected for soil analysis to determine total N, pH, P, K, organic matter, Ca, Mg, CEC, and base saturation. Total N was determined using a combustion N analyzer (CE NA-2000, Elantech Inc., Lakewood, NJ) employing the method of Dumas (Kirsten and Hesselius 1983). Other chemical analyses were conducted according to Recommended Chemical Soil Test Procedures for the North Central Region (of the United States): pH, Watson and Brown (1998); phosphorus, Frank et al. (1998); organic matter, Combs and Nathan (1998); potassium and other basic cations, Warncke and Brown (1998).

Statistical analysis

Analysis of variance for three depths, spatial distribution and the interaction between soil depth and spatial distribution was employed separately for each site. For 30-cm depth cores, a one-way analysis of variance was employed. Least significant difference (LSD) at a significant level of 0.05 was used to determine individual mean differences for treatments. Pearson correlation coefficients were employed to relate amino sugar-N and total N concentrations. For each chemical attribute at each location, one-way analysis of variance and LSD methods were employed. Software utilized was that of Release 9.1 of the SAS (Statistical Analysis System) Institute (2003).

RESULTS

Analysis of variance for amino sugar-N concentration beneath nitrogen-fixing *A. glutinosa* and nonnitrogen-fixing *T. americana* at the Arboretum site (Table 1) indicates significant differences among soil depths. The top 10 cm of soil had significantly higher levels of amino sugar-N than the deeper soil strata (Table 2). The tree canopy effect on soil amino sugar-N concentration for the core depth of 0-30 cm was significant for N-fixing *A. glutinosa* but not for the adjacent *T. americana* control (Tables 3 and 4).

Table 1. Analyses of variance for soil amino sugar nitrogen concentration (mg· kg^{-1}) around *A. glutinosa* trees and a *T. americana* tree at the University Arboretum.

Source of variation	**Degrees of freedom**	**Mean squares for amino sugar [N]**
<u>Near *A. glutinosa* trees</u>		
Soil depth	2	102764**
Canopy cover	1	5013
Depth × cover	2	1825
Error	78	2197
<u>Near *T. americana* tree</u>		
Soil depth	2	20149**
Canopy cover	1	2575
Depth × cover	2	1076
Error	15	725

** F value significant at the 1% level.

Table 2. Individual mean differences in amino sugar nitrogen concentration (mg· kg^{-1}) of soil from different depths associated with actinorhizal *A. glutinosa* trees and a *T. americana* tree at the University Arboretum.

Soil depth	**Number of samples**	**Mean amino sugar [N]**
<u>Near *A. glutinosa* trees</u>		
Depth 0-10 cm	28	424.3a[1]
Depth 10-20 cm	28	307.0b
Depth 20-30 cm	28	330.7b
<u>Near *T. americana* tree</u>		
0-10 cm	7	456.7a[1]
10-20 cm	7	368.1b
20-30 cm	7	362.8b

[1] Means with the same letter for a tree species are not significantly different (LSD, á= 0.05).

Table 3. Analysis of variance for soil amino sugar nitrogen concentration (mg· kg^{-1}) under or outside the canopies of actinorhizal *A. glutinosa* trees or a *T. americana* tree at a soil depth of 0-30 cm at the University Arboretum.

Source of variation	**Degrees of freedom**	**Mean squares for amino sugar [N]**
<u>Near *A. glutinosa* trees</u>		
Canopy cover	1	20877**
Error	41	1026
<u>Near *T. americana* tree</u>		
Canopy cover	1	431.71
Error	5	1050.82

** F value significant at the 1% level.

Table 4. Individual mean differences in amino sugar nitrogen concentration (mg· kg^{-1}) in the top 30 cm of soil associated with actinorhizal *A. glutinosa* trees and a *T. americana t*ree at the University Arboretum.

Canopy position	Number of samples	Mean amino sugar [N]
A. glutinosa trees		
Under the canopy	18	381.1a[1]
Away from the canopy	25	336.5b
T. americana tree		
Under the canopy	4	375.2a[1]
Away from the canopy	3	391.1a

[1] Means with the same letter for a tree species are not significantly different (LSD, á= 0.05).

A. glutinosa had 13% greater amino sugar-N concentration in soils under its crown at the University Arboretum compared with the soils outside the crown. The values for soil amino sugar-N at the Arboretum were higher than the N-fertilization nonresponsive value (>235 mg· kg^{-1} or 635 lbs per acre) in the top 30-cm-depth (Khan et al. 2001), yet increases in soil amino sugar-N, apparently due to nitrogen fixation, still occurred.

For DHNC, significant differences occurred for depths, but not for actinorhizal plant influence (Tables 5-8). The upper of 0-10cm of soil had significantly higher amino sugar-N concentrations than lower strata (Table 6). The mean values of amino sugar-N concentration for the stands that had been planted with N_2-fixing nurse trees were 316.1 mg· kg^{-1} (853 lbs per acre) and 309.7 (836 lbs per acre) mg· kg^{-1} for the control. Both mean values were greater than the N-fertilizer nonresponsive value (>235 mg· kg^{-1} or 635 lbs per acre) in the top 30 cm of soil (Khan et al., 2001). The high concentrations of soil amino sugar-N are associated with high natural fertility of the prairie-derived Mollisols.

Table 5. Analysis of variance for soil amino sugar nitrogen concentration (mg· kg^{-1}) at Douglas-Hart Nature Center.

Source of variation	Degrees of freedom	Mean squares for amino sugar [N]
Soil depth	2	176996**
Interplanting	1	177
Depth × treatment	2	573
Error	60	2360

** F value significant at the 1% level.

Table 6. Individual mean differences in amino sugar nitrogen concentration (mg· kg^{-1}) of different soil depths at Douglas-Hart Nature Center.

Soil depth	Number of samples	Mean amino sugar [N]
0-10 cm	22	440.8a[1]
10-20 cm	22	308.5b
20-30 cm	22	248.9c

[1] Means with the same letter are not significantly different (LSD, á= 0.05).

Table 7. Analysis of variance for soil amino sugar nitrogen concentration (mg· kg^{-1}) at a soil depth of 0-30 cm at Douglas-Hart Nature Center.

Source of variation	Degrees of freedom	Mean squares for amino sugar [N]
Interplanting	1	178.5
Error	20	1019.2

Table 8. Individual mean differences in amino sugar nitrogen concentration (mg· kg^{-1}) of stands interplanted or not with nitrogen-fixing trees at a depth of 0-30 cm at Douglas-Hart Nature Center.

Treatment	Number of samples	Mean amino sugar [N]
Interplanted	16	316.1a[1]
Noninterplanted	6	309.7a

[1] Means with the same letter are not significantly different (LSD, á= 0.05).

At Kickapoo State Park, amino sugar-N differed significantly both with soil depth and influence of nitrogen-fixing trees factors (Table 9). The upper 0-10 cm of soil had the greatest concentration of amino sugar-N, which declined sharply with increased depth. The greatest value in the upper fraction of soil was 253.8 mg· kg^{-1} (685 lbs per acre), which is more than the N fertilizer nonresponsive value of 235 mg· kg^{-1} or 635 lbs per acre. The 10-20 cm and 20-30 cm depths had significantly lower soil amino sugar-N concentration (Table 10). The factorial design yielded significant differences among nitrogen fixing tree and control treatment means that placed the *A. glutinosa* below controls in amino sugar-N concentration (Table 11). However, at a single soil sample depth of 0-30 cm, the actinorhizal *E. umbellata* and *A. glutinosa* plantings were both significantly higher in amino sugar-N concentration than controls, but not different from each other according to LSD mean comparisons (Tables 12 and 13).

Table 9. Analysis of variance for soil amino sugar nitrogen concentration (mg· kg^{-1}) at Kickapoo State Park.

Source of variation	Degrees of freedom	Mean squares for amino sugar [N]
Soil depth	2	230583**
Vegetative cover	2	12893**
Depth × cover	4	2537
Error	67	2521

** F value significant at the 1% level.

Table 10. Individual mean differences in amino sugar nitrogen concentration (mg· kg^{-1}) of different soil depths at Kickapoo State Park.

Soil depth	Number of samples	Mean amino sugar [N]
0-10 cm	26	253.8a[1]
10-20 cm	26	47.7b
20-30 cm	24	35.5b

[1] Means with the same letter are not significantly different (LSD, á= 0.05).

Table 11. Individual mean differences in amino sugar nitrogen concentration (mg· kg^{-1}) across depths of different vegetative cover at Kickapoo State Park.

Vegetative cover	Number of samples	Mean amino sugar [N]
E. umbellata	12	154.3a[1]
Grass or *S. interior*	10	127.6ab
A. glutinosa	54	103.1b

[1] Means with the same letter are not significantly different (LSD, á= 0.05).

Table 12. Analysis of variance for amino sugar nitrogen concentration (mg· kg^{-1}) for 0-30 cm core depths at Kickapoo State Park.

Source of variation	Degrees of freedom	Mean squares for amino sugar [N]
Vegetative cover	2	2721*
Error	22	541

* F value significant at the 5% level.

Table 13. Individual mean differences in amino sugar nitrogen concentration (mg· kg^{-1}) in the top 30 cm of soil for different vegetation at Kickapoo State Park.

Vegetative cover	Number of samples	Mean amino sugar [N]
E. umbellata	4	103.6a[1]
A. glutinosa	18	98.4a
Grass or *S. interior*	3	54.3b

[1] Means with the same letter are not significantly different (LSD, á= 0.05).

Soil chemical analysis

The two prairie Mollisols at the University Arboretum and Douglas-Hart Nature Center had higher mineral nutrient levels, greater organic matter percentages, greater total N, lower pH values, and higher cation exchange capacities than the mine spoils at Kickapoo State Park. The increases over controls in soil amino sugar-N concentrations under *A. glutinosa* trees at the University Arboretum were associated with lower pH and cation loss (Table 14). Soil properties at DHNC did not differ between treatments. Actinorhizal tree plantings at Kickapoo State Park greatly increased soil amino sugar-N concentration and were negatively correlated with total soil N concentration (Table 15).

Table 14. Chemical analysis of the surficial 30 cm of soil. CEC= cation exchange capacity. Values are means with standard deviation within parenthesis.

Source of soil	Amino sugar-N[1] (mg kg $^{-1}$)	TN[2] (mg kg $^{-1}$)	pH	P (mg kg $^{-1}$)	K (mg kg $^{-1}$)	Organic matter (%)	Ca (mg kg $^{-1}$)	Mg (mg kg $^{-1}$)	CEC[3] (cmol$_c$kg^{-1})	% Ca[4]	% Mg[4]	%H$^+$ [4]
University Arboretum (n=3)												
Beneath *A. glutinosa* canopy	381.4a[5] (4.8)	2050.0a (268.5)	4.9b (0.3)	31.67a (12.8)	198.83ab (63.1)	3.33b (0.3)	2073b (340.4)	367.7b (53.8)	23.9c (1.9)	43.13b (3.9)	12.77b (0.9)	42.00a (5.3)
Beyond *A. glutinosa* canopy	336.3b (5.7)	1766.7a (145.0)	5.5a (0.3)	33.50a (8.2)	260.17a (47.6)	3.33b (0.3)	2496b (116.1)	464.0b (15.9)	24.4cb (2.6)	51.30a (3.5)	15.90a (1.3)	30.00b (5.3)
Beneath *T. americana* canopy	383.3a (10.3)	2303.3a (1171)	5.6a (0.1)	25.0ab (2.2)	194.50ab (43.9)	4.0a (0.0)	3285a (297.4)	604.5a (68.3)	30.5a (3.0)	53.87a (1.2)	16.50a (0.8)	28.00b (2.0)
Beyond *T. americana* canopy	391.1a (46.1)	2183.3a (768.9)	5.6a (0.2)	16.33b (1.0)	143.50b (16.7)	4.0a (0.0)	3054a (255.2)	600.3a (53.6)	29.0ab (2.5)	52.77a (2.5)	17.27a (0.5)	28.67b (3.1)
Douglas-Hart Nature Center (n=3)												
Area interplanted with N_2-fixing trees	314.8a (3.9)	1473.3a (143.6)	6.1a (0.3)	18.00a (5.3)	203.83a (28.4)	3.67a (0.6)	4035a (540.1)	664.8a (242.2)	32.13a (4.6)	62.83a (1.6)	16.90a (4.4)	18.67a (5.0)
Area not interplanted with N_2-fixing trees	314.8a (2.9)	1346.7a (180.1)	5.7a (0.2)	11.83a (1.9)	175.50a (13.5)	3.17a (0.3)	3240a (82.5)	453.3a (58.0)	27.9a (2.1)	58.23a (4.9)	13.47a (0.8)	26.67a (4.2)
Kickapoo State Park (n=3)												
Beneath *A. glutinosa* trees	93.6a (1.4)	456.7b (80.8)	7.6a (0.1)	6.17a (0.8)	91.83b (10.7)	2.00a (0.0)	4124a (792.6)	437.8a (14.8)	26.2a (6.6)	84.5a (4.0)	14.57a (3.7)	0.00a (0.0)
Beneath *E. umbellata* shrubs	91.8a (3.0)	536.7ab (86.2)	7.3a (0.4)	4.17b (0.3)	130.83a (12.6)	2.83a (0.3)	3093a (1570)	413.5a (115.8)	19.4a (8.4)	78.0a (5.9)	18.73a (5.6)	1.30a (2.3)
From grass and *S. interior* stands	54.3b (8.0)	643.3a (110.2)	7.7a (0.1)	4.33b (0.8)	138.17a (26.6)	2.50a (0.9)	3399a (1285)	625.5a (167.1)	22.5a (5.0)	73.2a (14.2)	25.07a (13.4)	0.00a (0.0)

[1] Illinois Soil Nitrogen Test (Khan et al., 2001).

[2] Total nitrogen (Kirsten and Hesselius, 1983).

[3] Cation exchange capacity.

[4] Base saturation percentage of cation exchange sites.

[5] Means with the same letter within a column for a given location are not significantly different (LSD, á = 0.05).

Table 15. Pearson correlation coefficients (*r*) and P > F values for total N and amino sugar-N values.

	Total N		
	University Arboretum n=12	**DHNC n=6**	**Kickapoo Park n=9**
Amino sugar-N			
r	0.236	-0.266	-0.681
P > F	0.46	0.61	0.043

DISCUSSION

The spatial pattern of amino sugar-N accretion at the University Arboretum site suggests that its accumulation under the canopy of *A. glutinosa* trees results from nitrogen fixation. At the same site, with similar background N values, the nonnitrogen-fixing *T. americana* did not exhibit an increase over adjacent soils in amino sugar N under its canopy. The significant increase in soil amino sugar N under the *A. glutinosa* canopy occurred, even though soil amino sugar levels indicate high N fertility. High N fertility levels can inhibit symbiotic nitrogen fixation by plants, apparently through energy conserving feedback mechanisms (Binkley 1983; Fisher and Binkley 2000).

Amino sugar-N concentrations were always highest in the top 10 cm of soil consistent with the input of organic N from annual litter fall and surficial root dieback, but the degree of difference with respect to lower strata varied. The difference was greatest on the mine spoils where amino sugar-N concentration of the upper most 10 cm was 500% greater than the underlying 20 cm (8 inches) of soil, compared with about 60% greater surficial concentrations at DHNC, 30% greater around *A. glutinosa* at the University Arboretum, and 25% greater around *T. americana* at the University Arboretum. These differences may be proportional to the contribution of nitrogen-fixing trees to soil N fertility at the study sites. At DHNC, it is possible that an increase in amino sugar-N due to input from N-fixing trees has lost distinct spatial character with N cycling after death of most of the interplanted N-fixers. Also, there is more biomass in the interplanted stands at DHNC, which could constitute a major N pool for the site and explain the lack of significant differences in soil amino sugar-N.

According to LSD values of soil amino sugar-N in reclaimed mine spoils at Kickapoo State Park, *E. umbellata* had a significantly higher amino sugar-N concentration than *A. glutinosa* but not the control. In contrast, for a one-way ANOVA of the 0-30 cm depth, both *E. umbellata* and *A. glutinosa* had significantly higher amino sugar-N concentrations than the control plot. The analysis of results for the surficial 30 cm of soil indicated that the *E. umbellata* plot had the highest amino sugar-N, followed by the *A. glutinosa* plot and, lastly, the control plot. Consistent with these results, Paschke et al. (1989) found that the net N mineralization rates in an *Elaeagnus* interplanting were significantly higher than that in an *Alnus* interplanting. The difference in results of our study using different soil core volumes is likely owing to greater variability and sampling error associated with separate 10 cm core increments compared with one 30 cm depth soil core in heterogeneous, rocky mine spoils. None of the cover types on the mine spoils had an amino sugar-N value in the 0-30 cm depth soil cores exceeding the N-fertilization nonresponsive value for the same soil stratum of 235 $mg \cdot kg^{-1}$ for corn. This suggests that the site was deficient in soil N for maximum plant growth. The relative increase in soil amino sugar N by actinorhizal trees in the top 30 cm of soil was greater at the mine spoil site than in the two N-rich prairie Mollisols (Tables 4, 8, and 13). N-fixers probably increase nitrogen fixation rates on N deficient sites more than on N fertile sites because of feedback mechanisms that decrease energy demanding N-fixation and nodulation where substrate N is readily available (Fisher and Binkley, 2000). But nitrogen fixation sufficient to increase soil amino sugar-N apparently did occur even on a nitrogen-rich prairie Mollisol at the University Arboretum.

At the University Arboretum under *A. glutinosa* leaf canopies, there were significantly higher amino sugar-N concentrations, lower pH values and greater proton saturation of CEC exchange sites than beyond *A. glutinosa* canopies in the 0-30 cm soil layer. The 13% increase of amino sugar-N concentration beneath the *A. glutinosa* canopy is attributable to symbiotic nitrogen fixation by *A. glutinosa* trees. The decreased pH values beneath the *A. glutinosa* canopy can also be ascribed to added, biologically fixed N from *A. glutinosa* trees. Increased ammonium nitrogen from mineralization of litter and sloughed roots of *A. glutinosa* tissue enriched with fixed N is oxidized to nitrate via microbial nitrification, which can decrease soil pH and thereby increase cation leaching. Such a result is similar to previous findings for *A. rubra* on fertile soils (Binkley and Sollins 1990; Cole et al. 1990). Total N and amino sugar-N in soils were not correlated at this site (Table 15).

At Kickapoo State Park, the only significant correlation was negative (*r*= -0.681) and was found for the relationship between amino sugar-N concentration and total N. Higher amino sugar-N concentration may reflect easily mineralizable N associated with the actinorhizal plant littler and sloughed roots. The corresponding lower total N concentration suggests that the total soil N pool under N_2-fixing trees may have been reduced because of rapid mineralization. On control sites, low amino sugar-N concentrations and correspondingly high total N values suggest that mineralization may be slowed by immobilization of N with incorporation into soil of nonactinorhizal plant tissue possessing a higher C:N ratio and lower mineralization rates.

The higher phosphorus concentration beneath *A. glutinosa* at Kickapoo State Park is similar to findings of Giardina et al. (1995), who found that interplanted *A. glutinosa* trees enhanced soil inorganic P availability in soil. However, Spears et al. (2001) reported opposite results in which total soil phosphorus under actinorhizal *Ceanothus velutinus* Dougl. ex Hook was less than that under nonfixing species by 20%. Nitrogen-fixing species and soil types evidently have varied effects on the soil phosphorus pool.

In summary, nitrogen-fixing trees can significantly increase concentrations of ammonium and amino sugar-N in soils and also influence other soil properties such as pH, cation leaching, and soil phosphorus concentration. The results indicate that ammonium plus amino sugar-N concentrations estimated by the ISNT closely correspond with presence and density of nitrogen-fixing trees and may provide an accurate assessment of N-fixing trees’ contributions to labile soil organic N pools. The ISNT is apparently sensitive to increases in soil N fertility by N-fixing trees where other, more-complicated laboratory tests are not sensitive. Thus the test has potential as a site-specific index of their soil improvement capacities.

Apart from the discovery that the ISNT is sensitive to soil nitrogen fertility contributions by nitrogen-fixing trees, our findings have practical implications for the management of agroforestry systems with nitrogen-fixing tree components. The ISNT results clearly relate to actual soil N-fertility. The test itself is simple, precise and can be conducted anywhere with commonly-available and inexpensive materials. ISNT results should be able to guide the selection of appropriate tree and crop species, whether nitrogen-fixing or not, according to their N-fertility requirements or contributions. The test results, when calibrated with corresponding levels of productivity, should be able to predict the benefits expected from planting N-fixing trees on a particular soil type.

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