Quantifying Nitrogen Fixation of Agroforestry Shrub Species by the ^{15}N Natural Abundance and Dilution Techniques Under the Greenhouse Condition

G. Issah[1,3], J. D. Knight[1] , J. Kort[3] and A. A. Kimaro[1,2]

[1]Department of Soil Science, University of Saskatchewan
51 Campus Drive, Saskatoon, SK S7N 5A8 Canada. [2]Current address: World Agroforestry Centre, ICRAF-Tanzania Programme, Dar-es-Salaam, Tanzania
[3]Agriculture and Agri-Food Canada, Agroforestry Development Centre
P.O. Box 940 Indian Head, SK S0G 2K0

Key Words: nutrient management, agroforestry, isotopic methods, soil and water pollution

Abstract

The land use systems of the province of Saskatchewan has agroforestry trees such as sea buckthorn (*Hippophae rhamnoides* L.), caragana (*Caragana arborescens* Lam.) and buffaloberry (*Shepherdia argentea* Nutt.) as important components. They have been employed within the systems to perform functions such as ameliorating soil moisture, light and temperature through wind speed reduction and trapping snow. However, their N_2-fixation capability has not been determined. Therefore in a greenhouse experiment conducted at the Agriculture greenhouse at the University of Saskatchewan, Saskatoon, SK, Canada from January 2012 − April 2012, the N_2-fixation rates in the test species were evaluated to know their contributions to on-farm nitrogen management. After 120 days after planting, the shoot N derived from the atmosphere (%Ndfa) in the species ranged from 20-59%, 21-70% and 3-15% for caragana, sea buckthorn and buffaloberry, respectively. Also the whole plant %Ndfa in the species ranged from 18-47%, 49-54% and 38-41% for caragana, sea buckthorn and buffaloberry, respectively by both estimation methods. The contributions of the species to soil N were 73-91, 25-261 and 15-110 kg N ha^{-1} for caragana, sea buckthorn and buffaloberry, respectively by both methods. The amount was sufficient to meet the N requirements of most of the forage and grass species in the province which would progressively reduce the overreliance on synthetic fertilizers and minimize the risk of agrarian-derived soil and water pollution from inorganic nutrient sources.

Introduction

The growing global prominence on developing environmentally sustainable agroecosystems for food, fiber and fuel production, with less reliance on non-renewable resources coupled with the fact that N is the most limiting nutrient in most environments has made nitrogen (N_2)-fixing species gained importance (van Kessel and Hartley, 2000; Crews and Peoples, 2004; Franche et al., 2009; Hauggaard-Nielsen et al., 2010). This is because they have the ability to convert atmospheric N_2 to plant available N through symbiotic association with microbes.

Substantial amounts of N input from biological N_2-fixation (BNF) by trees or shrubs in symbiosis with microbes can yield positive effects for soil improvement and productivity of agroforestry and agricultural systems. The fixed N is released into the soil through litter-fall, root and nodule decay (Khanna, 1998). However, such inputs are subject to the effects of a number of variables including soil, climate, fixing tree-microbe interactions and management conditions (Mafongoya et al., 2004). In agricultural and agroforestry systems, N_2-fixing species can contribute substantially to the overall N economy of the system by sourcing atmospheric N via symbiotic N_2- fixation and through subsoil N retrieval (Gathumbi et al., 2002).

Despite the role played by trees and shrubs in the functioning of many ecosystems, from natural woodlands to plantations and agroforestry systems via BNF, it is difficult to quantify the amounts of N_2 fixed due to (a) diversity in occurrence, and large plant-to-plant variation in growth and nodulation status of N_2-fixing species (b) long-term, perennial nature of growth and the seasonal or year-to-year changes in patterns of N assimilation; and (c) logistical limitations of working with mature trees which are generally impossible to harvest in their entirety (Boddey et al., 2000). These setbacks make only few of the available techniques (isotope-based techniques) in estimating N_2-fixation applicable to deep rooted trees and shrubs in the field (Gathumbi et al., 2002).

The present study uses both of the isotope-based techniques (natural abundance and dilution techniques) in order to curtail the problem of undetectable $\delta\ ^{15}N$ natural abundance in the soil used. The hypothesis tested was that the symbiotic N_2-fixation rate by caragana would be higher than that in sea buckthorn and buffaloberry under greenhouse conditions. To test the hypothesis, the study investigated the symbiotic N_2-fixation rates by caragana, sea buckthorn and buffaloberry using both the ^{15}N natural abundance and the ^{15}N dilution techniques.

Materials and Methods

A greenhouse experiment was conducted in a growth chamber at the University of Saskatchewan, SK, Canada from January 2012 − April 2012 to quantify N_2-fixation using the ^{15}N natural abundance and the ^{15}N dilution techniques. Pots filled with a mixture of Brown Chernozemic soil and quartz sand in the ratio of 9:1, resulting into a bulk density of 1.10 g/cm^3 were arranged in a Completely Randomized Design (CRD) with six replications for each method, giving a total of 48 pots for both methods.

Treatments were three N_2-fixing tree species; caragana- the 'Ross strain'(*Caragana arborescens* Lam.), sea buckthorn- the 'Indian summer strain' (*Hippophae rhamnoides* L.) and buffaloberry

(*Shepherdia argentea* Nutt.) with choke cherry (*Prunus virginiana* var melancarpa L.) as the reference species. Prior to planting the seedlings, they were cold-hardened by storing in deep freezer for 90 days. The plants for the dilution method were labeled with ^{15}N-enriched NH_4^+-NO_3^- solution (10 atom % excess) at a rate of 5 kg/ha 30 days after the sprouting of seedling to allow for proper root establishment. The plants were grown for 120 days, equivalent to one growing season.

The shrubs were harvested 120 days after planting and partitioned into shoots, roots and nodules for N_2-fixing plants and shoots and roots for non N_2-fixing plant. The shoot and root materials were oven-dried at 60 °C to stable weight to determine dry biomass and subsequently ground to fine powder and sent for the analysis of total N concentration and ^{15}N isotope ratios on a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) coupled to a Delta V Advantage mass spectrometer (Thermo Scientific, Bremen, Germany) at the University of Saskatchewan (Saskatoon, SK, Canada).

The proportion of N in the agroforestry shrub species derived from the atmosphere (%Ndfa) was assessed by the ^{15}N natural abundance method using equation (1) and by the dilution method using equation (2) as detailed by Ledgard (1989) and Rennie and Dubetz (1986), respectively.

$$\%\text{Ndfa} = \left[\frac{\left(\delta\ 15\text{N}\ ‰_{\ non-fixing\ crop}\right)-\left(\delta\ 15\text{N}\ ‰_{\ fixing\ crop}\right)}{\left(\delta\ 15\text{N}\ ‰_{\ non-fixing}\right)-B}\right] \times 100 \quad \text{.....1}$$

The B values for the tested species were assumed to be 0 ‰ as done by Busse et al. (2007) because there are no published B-values for the tested species.

$$\%\text{Ndfa} = \left[1 - \frac{(\text{atom\%15N excess in fixing crop})}{(\text{atom\%15N excess in non-fixing crop})}\right] \times 100 \quad \text{.....2}$$

The amount of the N_2 fixed was calculated as detailed by Peoples et al. (2009)

$$\text{N fixed(kg/ha)} = \frac{\%\text{Ndfa}}{100} \times \text{legume N (kg/ha)} \quad \text{.....3}$$

$$\text{where legume N}\frac{\text{kg}}{\text{ha}} = \frac{\%\text{N}}{100}\text{x legume Dry Matter}$$

Whole plant and shoot amount of N_2-fixed and %Ndfa in the test species were analyzed using SAS 9.2 for Windows (SAS Institute, Cary, NC, 2008) using one-way analysis of variance (ANOVA) within the framework of a Completely Randomized Design at 5 % level of significance by the PROC MIXED procedure. Tukey's Honestly Significance Difference was used to separate treatment means when significant. The SAS macro pdmix800 was used to perform pairwise comparisons at 5% level of significance (Saxton, 1998).

Results

Nitrogen fixation capacity of tree/shrub species

There were significant differences in the estimated %Ndfa in roots (p = 0.0003) and shoots (p = 0.0007), however, whole plant %Ndfa was not significantly different (p = 0.8608) using the natural abundance method (Fig.1). Sea buckthorn had the highest %Ndfa value in all plant organs, followed by caragana and then buffaloberry. With the dilution method, %Ndfa in all the plant organs were significantly different among the test species: roots (p = 0.0037), shoots (p = 0.0260) and whole plant (p = 0.0066). Sea buckthorn had a higher shoot %Ndfa value followed by caragana and then buffaloberry. On whole plant basis, however, sea buckthorn had the highest %Ndfa followed by buffaloberry and then caragana (Fig. 2).

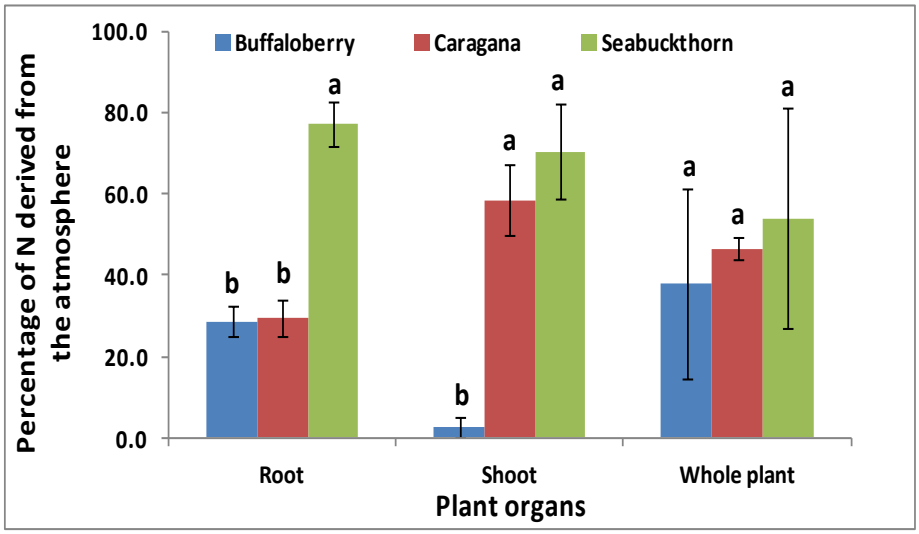

Fig. 1: %Ndfa among plant components by natural abundance method. Vertical bars show standard error of the mean (n=4). Means followed by same letters are not significantly different at p<0.05.

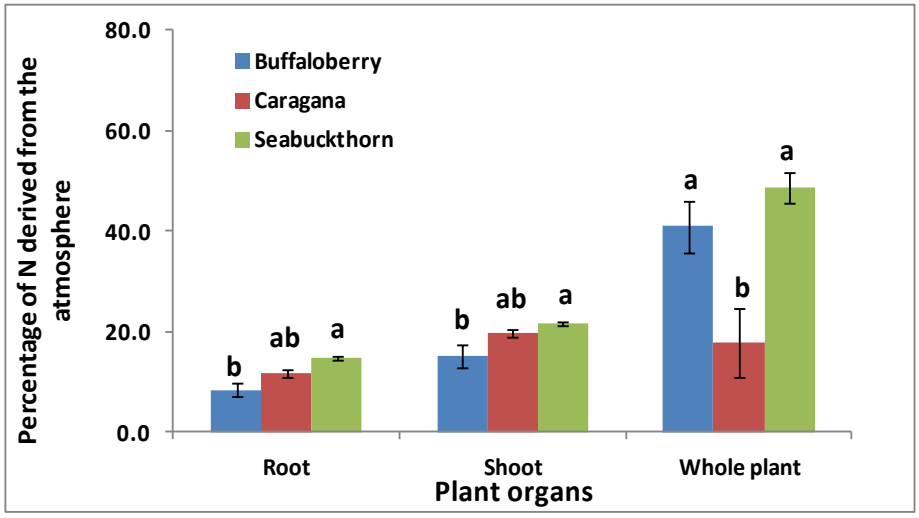

Fig. 2: %Ndfa among plant components by dilution method. Vertical bars show standard error of the mean (n=4). Means followed by same letters are not significantly different at $p<0.05$.

The contribution of tree/shrub species in N management

The proportion of N_2-fixed was significantly different among the test species: roots ($p = 0.0100$), shoots ($p = 0.0200$) and whole plant ($p = 0.0261$) by the natural abundance method (Fig. 3). Caragana had a higher amount of N_2-fixed in all the plant components. The whole plant N_2-fixed values were 0.46, 0.16 and 0.10 g N tree^{-1} for caragana, sea buckthorn and buffaloberry, respectively. By using the dilution method, the amount of N_2-fixed in all the plant components were also significantly different among the test species: roots ($p = 0.0057$), shoots ($p = 0.0124$) and whole plant ($p = 0.0107$) (Fig. 4). Sea buckthorn, however, had a significantly higher whole plant amount of N_2-fixed ($p = 0.0107$). The contribution of each species to the soil N pool on whole plant basis were 0.57, 1.64 and 0.69 g N tree^{-1} for caragana, sea buckthorn and buffaloberry, respectively.

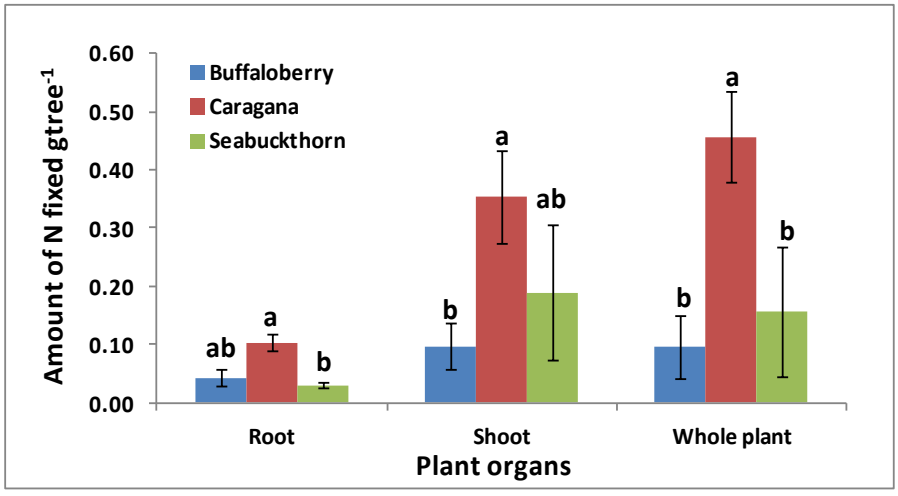

Fig. 3: Amount of N_2 fixed among plant components by natural abundance method. Vertical bars show standard error of the mean (n=4).

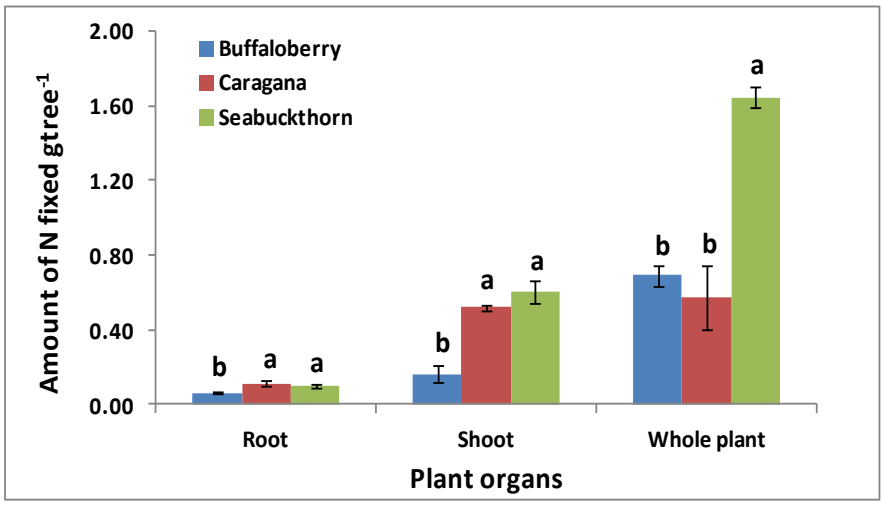

Fig. 4: Amount of N_2 fixed among plant components by dilution method. Vertical bars show standard error of the mean (n=4). Means followed by same letters are not significantly different at $p<0.05$.

Table 1: Whole Plant and Shoot %Ndfa and Whole Plant Amount of N_2-Fixed (Kg N/ ha) by ^{15}N Natural Abundance and ^{15}N Dilution Techniques after 120 Days of Planting

	%Ndfa				**N_2-fixed (kg N/ ha)**	
	Whole plant		**Shoot**		**Whole plant**	
Species	NA	EN	NA	EN	NA	EN
S. argentea Nutt. (**buffaloberry**)	38.0	41.0	3.0	15.0	15.0	110.0
C .arborescens L. (**caragana**)	47.0	18.0	59.0	20.0	73.0	91.0
H. rhamnoides L. (**sea buckthorn**)	54.0	49.0	70.0	21.0	25.0	261.0

Note: NA= Natural Abundance, EN= Enrichment (dilution)

Discussion

Both the whole plant and shoot %Ndfa values (Table 1) by natural abundance method generally conform to the results of Gathumbi et al., (2002) who found that %Ndfa in *Sesbania sesban* (L.) and *Calliandra calothyrsus* ranged from 46-59% and 36-54%, respectively 4 months after planting under the greenhouse conditions. Our results for the whole plant and shoot %Ndfa by dilution method (Table 1) are similar to whole plant %Ndfa in *Acacia leiocalyx* (25%) reported by Guinto et al., (2000) under greenhouse conditions using the ^{15}N dilution method.

The amount of N_2-fixed by all the test species using the dilution method and by caragana using the natural abundance method (Table 1) were more than the range of recommended N (56 kg N ha^{-1}) for grasses such as Timothy grass, smooth bromegrass and K-31 fescue grass (Angima et al., 2009) and therefore can satisfy the N requirements of the species to optimize growth. The study also proved that the amount of N_2 -fixed by the species using the dilution method can satisfy the N requirements of spring bread wheat crop for a normal season (i.e. 90 kg N ha^{-1}) as recommended by the (Conseil des productions végétales du Québec Inc., 1996; cited in Tran and Tremblay, 2000).

Conclusion

In summary, the greenhouse study showed that the test species can add a substantial amount of N to the soil N pool as demonstrated by the high amount of N_2-fixed (Table 1). Sea buckthorn recorded the statistically significant higher range of %Ndfa values using both estimation methods. These higher fixation in sea buckthorn translated into higher of N_2-fixed using the dilution method, however, caragana contributed significantly higher amount of N by the natural abundance method. The findings do not fully support the study hypothesis that symbiotic N_2-fixation rates would be higher in caragana than in sea buckthorn and buffaloberry. Since the amounts of N_2-fixed by all the test species using the dilution and by caragana by the natural abundance method are more than the range of the N requirements of many cool-season and forage crops, it shows that these shrub species can be good candidate species for incorporation into the existing land use systems within the Prairies.

Further studies are, however, needed to ascertain the fixation capabilities of the test species on the field and the effects of other parameters such as phosphorus, plant ontogeny, variety of species and moisture on the fixation capabilities. Also it is worth investigating to know the amount of the fixed N that is transferred to associated crops and how that affects their yields and nutrition.

Acknowledgements

Financial support from Agriculture and Agri-Food Canada-Agroforestry Development Centre, Indian Head under the Research Affiliate Program (RAP) is gratefully acknowledged. Further, the dedicated work of my supervisors; Diane J. Knight, Anthony A. Kimaro and John Kort is much appreciated.

References

Angima, S. D., R. L. Kallenbach and W. W. Riggs. 2009. Forage yield of selected cool season grasses in response to varying rates of nitrogen. Online. Forage and Grazing lands. doi:10.1094/FG-2009-0129-01-RS (Date Accessed October 28, 2012).

Boddey, R. M., M. B. Peoples, B. Palmer and P. J. Dart. 2000. Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. Nutr. Cycl. Agroecosyst. 57: 235-270.

Busse, M. D., M. F. Jurgensen, D. S. Page-Dumroese, and R. F. Powers. 2007. Contribution of actinorhizal shrubs to site fertility in a Northern California mixed pine forest. For. Ecol. Management 244:68-75.

Crews, T. E. and M. B. Peoples. 2004. Legume versus fertilizer sources of nitrogen: Ecological tradeoffs and human needs. Agric. Ecosys. Environ. 102: 279-297.

Franche, C., K. Lindström and C. Elmerich. 2009. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321: 35-59. doi: 10.1007/s11104-008-9833-8.

Gathumbi, S. M., G. Cadisch, and K. E. Giller. 2002. ^{15}N natural abundance as a tool for assessing N_2-fixation of herbaceous, shrub and tree legumes in improved fallows. Soil Biol. Biochem. 34:1059–1071.

Guinto, D. F., Z. Xu, A. P. N. House and P. G. Saffigna. 2000. Assessment of N_2-fixation by understorey acacias in recurrently burnt eucalypt forests of subtropical Australia using the ^{15}N isotope dilution techniques. Can. J. For. Res. 30: 112-121.

Hauggaard-Nielsen, H., L. Holdensen, D. Wulfsohn and E. S. Jensen. 2010. Spatial variation of N_2-fixation in field pea (*Pisum sativum* L.) at the field scale determined by the ^{15}N natural abundance method. Plant Soil 327:167–184. doi: 10.1007/s11104-009-0043-9.

Khanna P. K. 1998. Nutrient cycling under mixed-species tree systems in Southeast Asia. Agrofor. Syst. 38:99–120.

Ledgard, S.F., 1989. Factors affecting isotopic fractionation during nitrogen fixation by pasture legumes. In: Association Franćaise pour la Production Fourrage`re (Editors). Proc. XVI Int. Grassland Congress, I. Nice, INRA, France, pp. 123–126.

Mafongoya P. L., K. E. Giller, D. Odee, S. Gathumbi, S. K. Ndufa and S. M. Sitompul. 2004. Benefiting from N_2-fixation and managing rhizobia. In: Noordwijk M. van, G. Cadisch and C. K. Ong (eds) Below-ground interactions in tropical agroecosystems: concepts and models with multiple plant components. CABI International, Wallingford, pp 227–242.

Peoples, M. B., J. Brockwell, D. F. Herridge, I. J. Rochester, B. J. R. Alves, S. Urquiaga, R. M. Boddey, F. D. Dakora, S. Bhattarai, S. L. Maskey, C. Sampet, B. Rerkasem, D. F. Khan, H. Hauggaard-Nielsen, and B. S. Jensen. 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48: 1-17.

Rennie, R.J., and S. Dubetz. 1986. Nitrogen-15-determined nitrogen fixation in field-grown chickpea, lentil, faba bean, and field pea. Agron. J. 78: 654–660.

SAS Institute. 2008. SAS/STAT user's guide, vers. 9.2. SAS Institute Inc., Cary, NC.

Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Pages 1243-1246 in: 23rd SAS Users Group Intl., SAS Institute Inc., Cary, NC.

Tran, T. S. and G. Tremblay. 2000. Recovery of ^{15}N-labeled fertilizer by spring bread wheat at different N rates and application times. Can. J. Soil Sci. 80: 533–539.

Van Kessel, C. and C. Hartley. 2000. Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? Field Crops Res. 65: 165-181.