Determination of Inorganic Nitrogen in Native and Nonnative Plants by Mason Jar Diffusion

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Introduction

Invasion of exotic species is one of the most serious threats to native plant communities in North America. Exotic plants can disrupt normal ecosystem function by intercepting solar radiation, usurping mineral and water resources, and by chemically altering the soil environment. Midwestern prairie and woodland systems have evolved under conditions where nitrogen is limiting. The leaf litter of *Rhamnus cathartica* (European buckthorn), a serious invader of Northeastern Illinois oak woodlands, has been shown to contain high levels of nitrogen (Heneghan 2002), Once senesced, these rich litter resources are rapidly decomposed making excess mineralized nitrogen available for plant growth. This excess nitrogen can encourage additional invasion of exotic species, negatively impact soil microbial activity and cause leaching of nitrogen into aquatic systems. While limited work has been done on nitrogen content of buckthorn leaf litter (Heneghan 2002), little or no information is available on the nitrogen content of live leaves of buckthorn and other exotic woody plants in Midwestern oak ecosystems. Knowing the potential contribution of nitrogen from exotic species could be beneficial to oak woodland managers. Residual effects of nitrogen deposition, determined by degree and longevity of infestation, could influence the success or failure of restoration efforts. The purpose of this investigation was to determine the nitrogen content of live leaves of *R. cathartica*, in addition to other exotic woody plants, as compared to native species by means of a relatively new assay developed for soil nitrogen testing.

Materials and Methods

Plant Selection

Four of the eleven species of woody plants were chosen based on their invasive, non-native status. The remaining plants were selected because they represent the most common species found within the woodland being studied. Nitrogen concentrations of European Buckthorn leaves were of special interest due to its pervasiveness, and the suspicion that it plays a large role in nitrogen deposition within the woodland.

Apparatus

The nitrogen diffusion apparatus designed by R. L. Mulvaney was modified to determine the nitrogen content of live leaves. Modifications to the original diffusion unit are as follows:

- All stainless-steel parts were replaced with nylon parts of identical dimensions. The O-rings were replaced with silicone rubber caulking.
- The corner of the mounting base was ground down to accommodate the nut rather than notching the nut to fit the base.

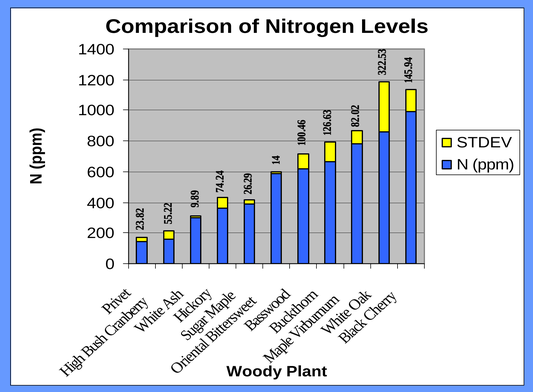
Reagents

A 4% boric-acid indicator solution was made:

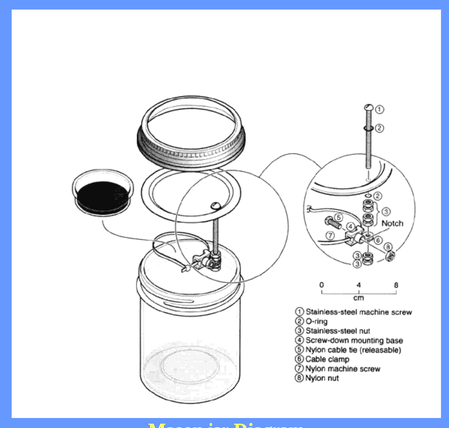
- Adding 40 g of reagent-grade H_3BO_3 to 1 L of deionized water.
- In a separate beaker, dissolve 0.495 g of bromocresol green and 0.333 g of methyl red in 20 mL ethanol and add to 980 mL of deionized water.
- 1 mL of this solution was added to the H_3BO_3 solution. The pH of this solution was adjusted to 4.8 - 5.0 by adding a small amount of granulated NaOH.

Procedures

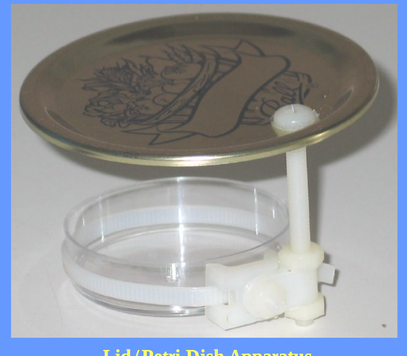
- Add 1 g of fresh minced leaves into the bottom of the Mason jar.
- Add 5 mL of H_3BO_3 indicator solution to the Petri dish (held in place by cable tie).
- Add 10 mL of NaOH to the minced leaves, and gently swirled mixture.
- Add lid containing assembly and Petri dish, and tightly secure lid with screw band.
- Allow a minimum of 40 hours for complete diffusion to occur.



Graph 1



Mason jar Diagram With Part List



Lid/Petri Dish Apparatus

Results

The average fresh leaf nitrogen concentrations obtained from the Mason jar diffusion range from 149 ppm for Privet to 992 ppm for Black Cherry with Buckthorn at 666 ppm (Graph 1). Due to endpoint variability, a slight variation of color change could result in an average error range of as much as +/- 90 ppm.

Conclusions

The Mason jar diffusion method, originally developed to determine the nitrogen concentration of soil, promises to be a reliable technique for establishing the N content of fresh leaves. However, the preliminary results of this investigation do not support the hypothesis that the fresh leaves of buckthorn contain a higher N content than all native species tested. Although oak and black cherry show higher N levels, it should be noted that nitrogen content from fresh leaves do not represent nitrogen deposition from leaf litter.

Further testing is needed to develop certain standards such as a set digestion time per assay, and a consistent titration endpoint. Also, it is necessary to identify the best time of the growing season to obtain leaves to get the highest nitrogen level per species. For example, the total nitrogen content of the sugar maple declines throughout the growing season (Schultz et al., 1982), and in early autumn, it is reputed to form an abscission layer across the phloem elements at the base of the petiole preventing nutrients from being recovered by the plant. On the contrary, Oaks reabsorbed leaf nutrients and stored them in their trunks (Curtis, 1959). Once these standards have been established, samples should be run for comparison purposes under lab conditions using either High Performance Liquid Chromatography or the Carlo Erba C/N analyzer.

Factors that may have had a direct impact on the ppm outcome in this trial run were the accuracy of the molarities of chemicals being used for both digestion and titration, the correct mixture of indicator being used, and the existence of contamination within the jar and Petri dish.

When all these criteria have been met, the Mason jar diffusion test may be considered a viable method for determining the nitrogen content of fresh leaves.

References

Chicago Wilderness Magazine (Fall 2002) News of the Wild.“Invasive Buckthorn Reduces Forest Leaf Litter”. http://chicagowildernessmag.org/issues/fall2002/news/buckthorn.html (Accessed 13 Aug. 2004)

Curtis, J.T.(1957) The Vegetation Of Wisconsin: An Ordination of Plant Communities. 2nd Ed., p.108. The University of Wisconsin Press, Wisconsin.

Gale, S.W. “Control of the Invasive Exotic *Rhamnus cathartica* in Temperate North American Forests” http://www.hort.agri.umn.edu/h5015/00papers/gale.htm (Accessed 13 Aug. 2004)

Khan, S.A., Mulvaney R..L., Mulvaney C.S., and Stevens W.B. (1997) “Simple Diffusion Methods To Determine Inorganic Nitrogen In Soil And Water” http://frec.cropsci.uiuc.edu/1997/report11/ Illinois Fertilizer Conference Proceedings. University of Illinois at Urbana-Champaign. (Accessed 13 Aug. 2004

Schultz, J.C., Nothnagle, P.J., Baldwin, I.T. (1982) Seasonal And Individual Variation In Leaf Quality Of Two Northern Hardwoods Tree Species, American Journal of Botany 69(5): 753-759.