Nitrogen dynamics of hairy vetch (Vicia villosa) and red clover (Trifolium pretense L.) legume cover crops in organically-managed agroecosystems in the northern United States

Dinámica del nitrógeno de los cultivos de cobertura de leguminosas vezo piloso (Vicia villosa) y trébol rojo (Trifolium pretense L.) en los agroecosistemas manejados orgánicamente en el norte de los Estados Unidos

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Abstract

Cover crops, non-harvested crops grown between cash crop production, are an agroecological approach that can increase ecosystem service provisioning. Legume cover crops in particular are valuable for their contribution of nitrogen (N) via the process of biological nitrogen fixation. Organic producers in the United States are prohibited from utilizing synthetic nitrogen fertilizer, and thus employ legume cover crops to provide available N for their non-legume cash crops. This study evaluates the potential of legume cover crops to contribute to soil N pools. We assessed four winter annual cover crop treatments at two sites in the state of Minnesota in the northern United States, including a rye non-legume control (*Secale cereal,* RYE), red clover (*Trifolium pretense L*., CLO), hairy vetch ecotype 1 (*Vicia villosa,* V1), hairy vetch ecotype 2 (V2), and a hairy vetch – rye bi-culture (V2 MIX). Additional treatments of with-rhizobia inoculation and without-rhizobia inoculation were imposed to determine the need for inoculation. Results showed that cover crop legumes contributed from 30-75 kg N ha yr-1, with 50-70% of plant biomass N derived from the atmosphere via biological nitrogen fixation. An increase in available soil N was observed following spring cover crop termination. No differences were observed in nitrogen fixation or nodulation parameters, suggesting that sufficient native soil rhizobia populations were present to carry out nitrogen fixation.

Keywords: organic agriculture, nitrogen fixation, rhizobia, legume, soil

Resumo

Los cultivos de cobertura son cultivos no cosechados, los cuales son sembrados entre cultivos comerciales, y son un manejo agroecológico que aumenta los recursos del ecosistema. Los cultivos de coberturas de leguminosas son valiosos por su aporte de nitrógeno (N) a través del proceso de fijación biológica de nitrógeno. Para los productores orgánicos en los Estados Unidos está prohibido aplicar fertilizantes sintéticos, así los cultivos de cobertura de leguminosas son importantes para proporcionar N a los cultivos comerciales que no son leguminosas. Este estudio evalúo el potencial de los cultivos de cobertura de leguminosas para aportar N en el suelo. Cuatro tratamientos de cultivos de cobertura de leguminosas fueron sembrados en dos estaciones experimentales en el Estado de Minnesota, en el norte de los Estados Unidos, incluyendo un control no-leguminosa de centeno (Secale cereales, RYE), trébol rojo (Trifolium L. pretensión, CLO), vezo piloso ecotipo 1 (Vicia villosa, V1), vezo piloso ecotipo 2 (V2), V2 + centeno (V2 MIX). Tratamientos adicionales de la inoculación con rizobios (WIN) y sin rizobios (NIN) fueron usados para determinar la necesidad de la inoculación. Los Resultados mostraron que los cultivos de cobertura contribuyeron entre 30-75 kg N ha-1 yr-1, y entre el 50-70%

de N de la biomasa vegetal se obtuvo de la atmósfera a través de la fijación biológica de nitrógeno. Un aumento de N fue observado en el suelo después de la terminación de las leguminosas. No se observaron diferencias en la fijación de nitrógeno o parámetros de nodulación, lo que sugiere suficientes rizobios nativos en el suelo para llevar a cabo la fijación de nitrógeno.
Palabras-clave: agricultura orgánica, fijación de nitrógeno, Rhizobium, vegetal, suelo

Introduction

Agroecosystem challenges facing northern regions of the United States include short growing seasons and climate variability. Cover crops, non-harvested crops grown between cash crop production, are an agroecological approach that can increase ecosystem service provisioning, with benefits of erosion reduction, spatial and temporal biotic diversification, nutrient additions, and increased soil organic matter (Parr et al., 2011). Soil organic matter influences soil structure, water retention, and plant available nitrogen (N) made available through microbial mineralization processes (Giacometti et al., 2013). Leguminous cover crops increase soil N by converting atmospheric N into plant soluble nitrogen, and may also increase organic carbon (C; Marriot and Wander, 2006). Soil C can be measured via assays that fractionate organic matter into pools that are considered to be stable, and those that are labile and thus accessible to microbes for nitrogen cycling. We will report on two years of experimental data at two sites in Minnesota, with objectives to: 1) assess viability of fall-planted, winter annual legume cover crops in the upper Midwest, 2) quantify nodulation and nitrogen-fixation capacity of legume cover crops, and 3) quantify cover crops contribution to soil N pools.

Methods

In September of 2014 and 2015, winter annual cover crop species including a rye non--legume control (*Secale cereal,* RYE), red clover (*Trifolium pretense L*., CLO), hairy vetch ecotype 1 (*Vicia villosa,* V1), hairy vetch ecotype 2 (V2), and a hairy vetch – rye bi-culture (V2 MIX; 2015 only), were sown. In the legume crops, additional treatments including with-rhizobia inoculant (WIN) and no-rhizobia inoculant (NIN) were also imposed. Trials were planted at two research stations in Minnesota, in the warmer, agronomic southwest region (Lamberton) and colder, forested northern region (Grand Rapids). For soil C, N, and microbial measurements in 2015, all treatments were compared with a bare-ground control.

In both years, cover crops were sampled in early June to obtain accurate biomass N concentration at cover crop termination. Samples were cut to ground-level using four 0.5 m^2 quadrats/treatment, dried at 65ºC, ground at 2mm, and analyzed using an Elementar™ vario PYRO cube to obtain total C/N and isotopic ratios. For biological nitrogen fixation (BNF) measurements, the ^{15}N natural abundance method, based on isotopic fractionation, was used to determine proportion of legume nitrogen derived from the atmosphere (Ndfa), also prior to cover crop termination. The impact of inoculation at cover crop planting was assessed by collecting subsamples of field plants from both WIN and NIN treatments and quantifying nodulation and rhizobia genetic structure via 16S-RNA sequencing approaches.

In year two we assessed cover crop contribution to soil carbon and nitrogen pools in June before cover crop termination and two weeks after termination, in order to capture soil C and N contributions from cover crop residue. Potentially mineralizable nitrogen (PMN) is the amount of nitrogen converted from organic to mineral forms during a 7-day, anaerobic, laboratory incubation and subsequent KCl soil N extraction. This is an important proxy for determining the capacity of the soil to provide nitrogen in forms available to plants, especially when employing organic inputs.

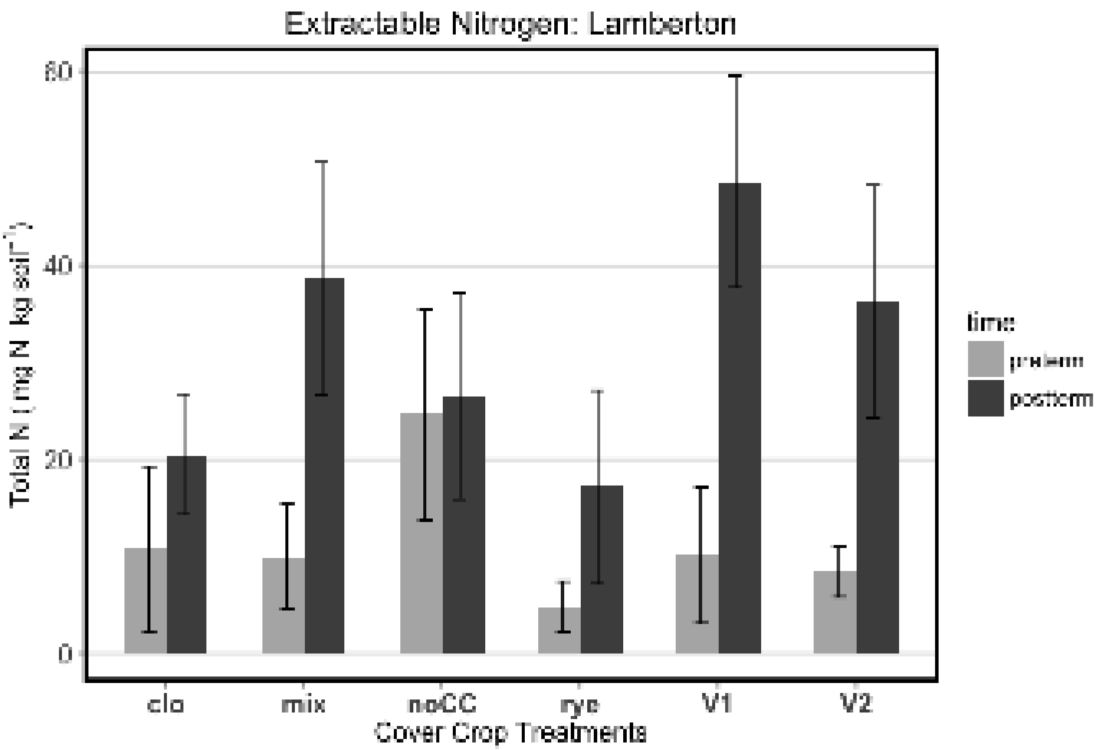
Results and Discussion

Inoculation did not have a significant effect on plant biomass N, nodulation, or genetic structure of rhizobia occupying nodules in either year, with no differences observed between legumes WIN and NIN treatments. Preliminary isotopic data from ^{15}N natural abundance assays in Lamberton indicated efficient nitrogen fixation, with approximately 50-70% of legume plant N derived from the atmosphere across all evaluated legumes (Table 1.). These data suggest native field rhizobia populations were sufficient to nodulate legume cover crops of interest, and fix adequate atmospheric nitrogen.

Table 1. Mean percent of total legume plant biomass Nitrogen derived from the atmosphere (Ndfa) measured via Natural abundance isotopic analysis.

Treatment	% Ndfa (se)
Trifolium pretense L., (CLO)	60.4 (3.2)
Vicia villosa (V1)	60.7 (5.4)
Vicia villosa (V2)	52.8 (4.3)
Vicia villosa – rye (*Secale cereal*) bi-culture (V2 MIX)	67.9 (2.4)

Higher overall legume biomass was observed in year two compared to year one, contributing to soil N and C pools. Year one planting failed to produce biomass due to poor establishment from late planting. At Grand Rapids, both potentially mineralizable soil N (data not shown) and extractable soil N increased following termination in all treatments, including the bare-ground control plots (Figure 1). However, in Lamberton, extractable N only increased in cover cropped plots following termination relative to the control plots, and PMN decreased compared to pre-termination levels. The increase in extractable N across legume, non-legume, and control plots at both sites suggests a complex relationship governing N contribution to soil pools following cover crop termination, likely due to factors including tillage intensity, plant inputs, and soil moisture. The increase in soil N on legume and rye cover cropped plots relative to bare-ground plots in Lamberton suggest that this increase was indeed due to the addition of N-rich legume and substantial residue at termination. However, at Grand Rapids, all plots including the bare-ground control showed increased soil N following cover crop termination. Tillage required to terminate and incorporate legume residue, which occurred across all treatments, may have been responsible for soil N increases and not cover crop residue additions, as no legume biomass was contributed on these plots. Previous research indicates tillage can serve to mineralize N protected in SOM rich soil aggregates and thus protected organic N may accumulate in the absence of tillage (Larsen et al., 2014; Six et al., 2002). The Grand Rapids site had been perennially-cropped in orchard grasses for over ten years prior to our trial, and thus may had significant organic N sequestered in protected organic matter pools. This is supported by overall greater PMN and available N at Grand Rapids compared to the Lamberton site.



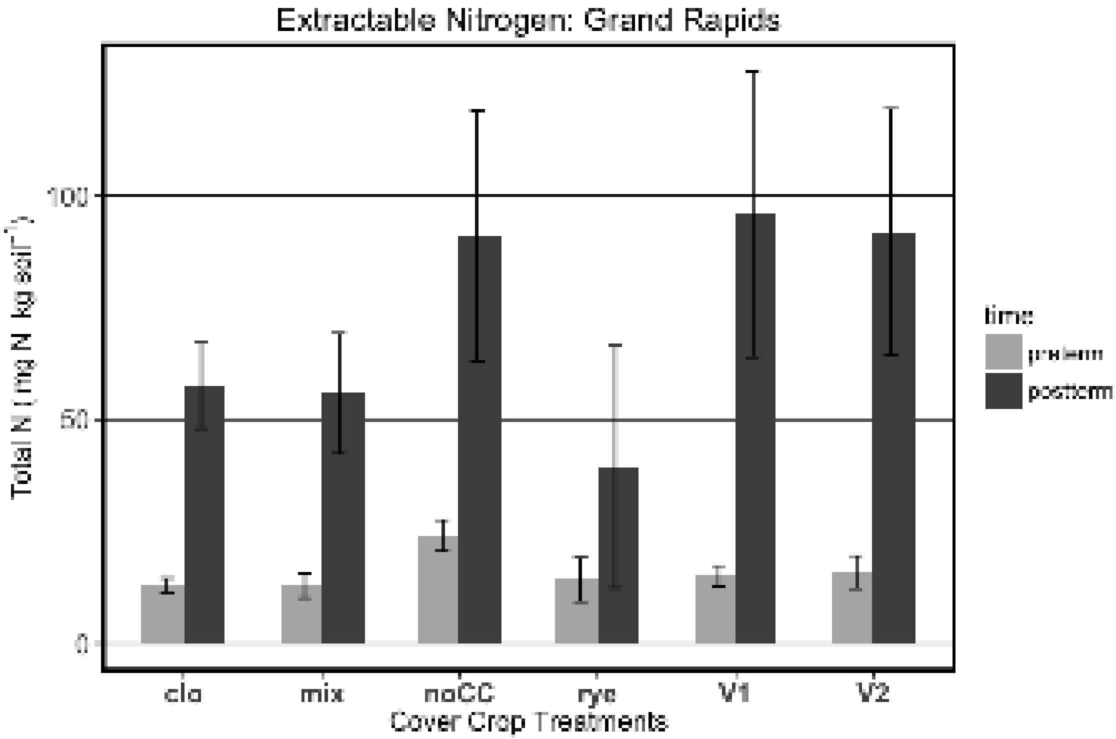


Figure 1. KCl extractable nitrogen (N) before cover crop termination (preterm) and following cover crop termination (postterm). Treatments included CLO, MIX, RYE, V1 and V2, compared to a bare-ground control (noCC). Top figure data taken at Lamberton, Minnesota, U.S.A. and bottom from Grand Rapids, Minnesota, U.S.A.

Conclusions

Cover crop legumes overwintering in northern regions of the United States have the capacity to contribute significant N to organic agroecosystems, provided they are actively fixing nitrogen, and have sufficient growing degree days to accumulate biomass N. In fields that have been out of production for many years, organic N may be protected in soil organic matter, and tillage events required for cover crop termination and soil incorporation in the spring may provide additional available N via mineralization of these protected pools. To improve coupled C and N cycling and predictive N availability, further research is needed to understand the interaction between N inputs from annual legumes and long-term N mineralization from organic pools. Farmers may seek to occupy unutilized times of the season for production of legume cover crops, or planting legumes in association with cash crops.

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