Nitrogen cycling in the cranberry agroecosystem: The importance of ericoid mycorrhizal fungi and organic nitrogen pools

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Nitrogen cycling in the cranberry agroecosystem: The importance of ericoid mycorrhizal fungi and organic nitrogen pools

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We investigated ericoid mycorrhizal (ERM) symbiosis, resorption rates, as well as leaf litter quality and decomposition to infer nitrogen (N) conservation strategies in cultivated cranberry plants. Resorption rates (35.7 ± 3.5 % [s.e.]) were moderate, and the resulting cranberry leaf litter had high C:N ratios (56.9 ± 0.02). Decomposition was slow with 57 ± 2 % of initial leaf litter mass remaining at the end of 2.5 years. The negative $\delta^{15}N$ values of cranberry leaves (-2.61 ± 0.27 0/00) and positive $\delta^{15}N$ values of cranberry roots (1.04 ± 0.35 0/00) provided evidence that N uptake was mediated by ERM fungi. We demonstrated that even in this intensively managed agroecosystem, cranberry plants retain N conservation strategies.

To infer cranberry and ERM fungi use of organic N sources, we measured ERM colonization, and the main inputs, outputs, and N pools in three cultivated cranberry beds. Inorganic N fertilizer was the dominant form of N added to the system (40 kg N ha^{-1} y^{-1}), but DON dominated soil N pools. Approximately 10% of total N from biomass was removed each year with harvest, but a similar amount of N was recycled back into the system through stem and litterfall. Overall, our results showed that net N inputs exceeded

net N outputs, indicating that cranberry beds were a sink for N. There is also potential for organic N as a nutrient source.

Ericoid mycorrhizal colonization and extractable soil N pools were measured in five cultivated cranberry beds in Central Wisconsin to determine if nutrient status of cranberry beds had a significant relationship on ERM colonization. Ammonium and nitrate concentrations were negatively correlated with ERM colonization, while DON soil pools exhibited a positive relationship. There was no significant relationship between ERM colonization rates and fruit yield or leaf N content.

The results of this study will be used to develop recommendations to growers for better nutrient management.

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Introduction to dissertation

Rationale

Nitrogen (N) is considered a limiting nutrient in the cranberry *(Vaccinium macrocarpon* Ait.) agroecosystem, and growers regularly apply fertilizer to meet yield requirements and remain in production. However, there are large gaps in the overall understanding of nutrient cycling in commercially cultivated cranberry beds. Growers are required to comply with state-mandated farm nutrient management plans, which account for nutrient fluxes from production. Therefore, there is widespread interest among growers to reassess nutrient management to meet yield goals, while also applying minimal amounts of fertilizer that are expensive and prone to aqueous runoff, leaching, or atmospheric loss.

A better understanding of N cycling in the cranberry agroecosystem will enable growers and policy makers to make informed decisions about future N management practices. We applied an agroecological approach to conducting this study, since we aimed to identify key biotic factors that operate within the cranberry agroecosystem that might be used to promote N persistence and enhance sustainability of production. This research project was a descriptive study that focused on exploring two key aspects of N cycling in the cranberry agroecosystem, the influence of ericoid mycorrhizal fungi and the importance of organic N pools as plant nutrient sources.

Background Information

Organic N pools are comprised of a wide range of material, from leaf litter to soil organic matter during in a continuum of decomposition. This includes dissolved organic N (DON) in the soil solution. We have reason to believe that the pool of organic matter may be high in cultivated cranberry beds because decomposition is slow. Through a symbiotic relationship with ERM fungi, cranberry plants have access to N not only from mineral ions, but also from substrates within their environments, such as leaf litter or DON pools in the soil solution. This type of symbiotic relationship is usually found between plants and fungi in low N ecosystems, such as heathlands and the arctic tundra. We sought evidence that this relationship also exists under fertilized conditions of cranberry agroecosystems.

Organic N pools are naturally replenished by leaf litter additions, fine root turnover, and decomposition processes throughout the season. Further research on the cranberry-ERM fungal association may provide growers with important information that may help them to take advantage of an ecosystem service, namely the ability of plants to use DON. Our research was based on cranberry production in Central Wisconsin, but the findings should be relevant to other production areas in North America (including Massachusetts, New Jersey, Oregon, Washington, British Columbia, Quebec and Montreal). Understanding N management with cranberries has implications for management of other nursery crops, including blueberries and rhododendrons, which are closely related ericaceous species that are known for their ERM symbioses.

Objectives

Prior studies have documented that ericoid mycorrhizal fungi are ubiquitous in cultivated cranberry beds in Wisconsin, but growers do not manage for them nor do they know the potential benefits that they may provide to production. Therefore, we wanted to establish a relationship between ERM fungi and N cycling. Additionally, inorganic N fertilizer is carefully monitored and applied, but it the amount of N contained in organic N pools has not been previously documented. Because of their symbiotic relationship with ERM fungi mobilize N, these may be significant nutrient sources for cranberry plants. To our knowledge, no prior studies have investigated the relationship between cranberry plants, ERM fungi, and organic N sources in a field setting with fertilizer applications.

The aim of the study presented in Chapter 1 was to evaluate whether N cycling in the cranberry agroecosystem resembled that in native ecosystems dominated by ericaceous plants. The characteristics that were explored were high N retention in litter and organic matter, uptake of fertilizer N, and possible mobilization of N from organic material by mycorrhizal fungi. The methods used to explore this topic were litter incubation in the field, analysis of N pools, and ^{15}N natural abundances.

Since one of the findings in Chapter 1 stated that ERM fungi were actively mobilizing N from the soil solution to the plant, Chapter 2 focused on creating an N budget in an attempt to infer use of organic N sources by ERM fungi. Towards that end, we measured ERM colonization, and the main inputs, outputs and nitrogen pools in cultivated cranberry beds.

Since first two chapters focused on the importance of ERM fungi in mediating N cycling in cultivated cranberry beds, Chapter 3 focused on factors that may influence ERM colonization rates in the field. The objective of the study was to determine if soil N pools were significantly correlated with ERM colonization rates. Higher rates of ammonium have been known to suppress ERM colonization. A deeper understanding of these issue could have important implications for management, as ERM fungi increase uptake and absorption of essential nutrients, which reduces N losses to groundwater, atmosphere, and downstream aquatic ecosystems.

Chapter 1. Nitrogen conservation strategies of cranberry plants and ericoid mycorrhizal fungi in an agroecosystem

Abstract

Ericoid mycorrhizal fungi (ERM) are ubiquitous in cultivated cranberry beds, but no prior studies have used ^{15}N natural abundance measurements to track ERM influence on nitrogen (N) cycling in this agroecosystem. Cranberries (*Vaccinium macrocarpon*) evolved in low-nutrient peat bogs, and ERM symbioses often occur in nutrient-poor environments where N is limiting. We investigated ERM symbiosis, resorption rates, as well as leaf litter quality and decomposition to infer N conservation strategies in cultivated cranberry plants. We expected moderate to high resorption rates and low leaf litter decomposition rates. We also expected to find evidence of ERM fungi mediating N uptake. Resorption rates (35.7 ± 3.5 % [s.e.]) were moderate, and the resulting cranberry leaf litter had high C:N ratios (56.9 ± 0.02). Decomposition was slow with 57 ± 2 % of initial leaf litter mass remaining at the end of 2.5 years. The negative $\delta^{15}N$ values of cranberry leaves (-2.61 ± 0.27 $^{0}/_{00}$) and positive $\delta^{15}N$ values of cranberry roots (1.04 ± 0.35 $^{0}/_{00}$) provided evidence that N uptake was mediated by ERM fungi. We demonstrated that even in this intensively managed and fertilized agroecosystem, cranberry plants retain N conservation strategies observed in wildland cranberry ecosystems, namely high resorption efficiencies, low-quality leaf litter, and active ERM symbiosis. This study has management implications, since current nutrient plans mainly focus on inorganic N fertilizer regulation and do not recognize the role of ERM fungi in mediating N uptake.

Keywords:

Ericoid mycorrhizal symbiosis, *Rhizoscyphus* (syn. *Hymenoscyphus*) *ericae*, *Vaccinium macrocarpon* (cranberry), Litter decomposition, Resorption, ^{15}N natural abundance, Nitrogen cycling

Introduction

Native cranberries evolved in low nutrient temperate bogs (Eck, 1990), yet cultivated cranberries (*Vaccinium macrocarpon* cv. Stevens) grow in a fertilized agroecosystem and receive considerable additions of inorganic N fertilizer (~40 kg N ha^{-1} y^{-1}). Cultivated cranberry may still exhibit some nitrogen (N) conservation strategies that may reduce the need for expensive N fertilizer that is prone to aqueous runoff or atmospheric loss. Plant ecology theory posits that plant species in nutrient-poor environments have higher nutrient resorption efficiencies than species from nutrient-rich sites (Aerts, 1996). However, evergreen species, such as cranberry can reduce N losses by producing leaves with a long life span and low nutrient concentrations, thus reducing the importance of high resorption efficiencies (Aerts, 1996; Eckstein et al., 1999). Evergreen plants also produce low quality leaf litter with high C:N ratios, which decomposes slowly (Berendse, 1994; Chapman et al., 2006). This conserves nutrients proximal to the plant root, making them easily accessible for plant uptake. Finally, symbiotic association with mycorrhizal fungi is another N conservation strategy found in plants growing in nutrient poor systems (Aerts et al., 2000; Chapman et al., 2006). The symbiosis enables plants to access more nutrients, including complex organic N forms

from decomposing organic material (Bajwa and Read, 1985; Bending and Read, 1996; Cairney and Burke, 1998; Read and Perez-Moreno, 2003).

Cranberry, a low-growing evergreen vine, grows in soils that are different from most agronomic and horticultural crops. The top 10 to 15 cm of cranberry soils at our research sites are characterized by distinct alternating layers of organic matter (OM) and sand because Wisconsin growers apply 1 to 2.5 cm of sand to the beds every 3 to 5 years in the winter. Organic matter layers are made of mostly leaf litter, with some stem and root material. These layers are usually aerobic during the growing season, since it is common for growers in Wisconsin to flood only for fall harvest and to form ice for overwinter frost protection.

We established a study to investigate the role of three N conservation strategies, resorption efficiency, leaf litter decomposition, and mycorrhizal symbiosis in the fertilized cranberry agroecosystem. Aerts (1996) found in a survey of evergreen species from Europe and the USA resorption efficiencies were less than 50% of leaf N. Fertilization appears to influence resorption, as the resorption efficiency of the evergreen ericaceous species, Empetrum hermaphroditum, fell from about 55% in natural bog conditions to about 40% when 100 kg N ha^{-1} of fertilizer was added annually for four years (van Heerwaarden et al., 2003). Because cranberry plants are also evergreen and grow in fertilized conditions, we expected resorption efficiencies to be below 50% of leaf N (Figure 1.1, pathway a).

We expected low leaf nutrient concentrations accompanied by moderate to high resorption efficiencies would contribute to high C:N ratios in leaf litter, which would

slow decomposition and mineralization in the cranberry beds (Figure 1.1, pathway b). Therefore, we expected that through their symbiosis with ericoid mycorrhizal fungi (ERM) cranberry plants would mobilize N from fertilizer or possibly organic N directly from decomposing leaf litter (Figure 1.1, pathway c). We considered the leaf litter possible N source since it has been demonstrated in lab studies that ERM fungi can access complex forms of N (Kerley and Read, 1995). Ericaceous plants commonly form associations with ericoid mycorrizhal fungi (ERM) such as *Rhizoscyphus* (syn. *Hymenoscyphus*) *ericae* (D.J. Read), W.Y. Zhuang and Korf) (Read and Perez-Moreno, 2003; Read et al., 2004). Ericoid mycorrhizal fungi have been found throughout cultivated cranberry beds in Oregon and Wisconsin (Kosola and Workmaster, 2007; Scagel, 2005). Evidence of both endo- and ectomycorrhizal fungi mobilizing N for plant uptake can be determined by comparing the $\delta^{15}N$ values of plant leaves and roots (Högberg et al., 1996, Emmerton et al., 2001, Hobbie and Hobbie, 2006). These studies found ^{15}N enrichment of fungal tissue relative to plant tissue, especially leaves. During the transfer of N from fungal hyphae to the plant, the mycorrhizae became enriched with the heavier isotope. Since cranberry roots contained fungal hyphae of ERM, we expected cranberry root tissue to be enriched with ^{15}N relative to cranberry leaves.

The importance of ERM fungi mediating N uptake has been relatively well documented in unmanaged systems such as heathlands (Read et. al., 2003), boreal forests (Lindahl et al., 2007), and the arctic tundra (Michelson et al., 1998; Hobbie and Hobbie, 2006). Here we described a field study designed to test the hypothesis that in a fertilized agroecosystem, cultivated cranberry plants display N conservation strategies, including ERM symbiosis. This may be important for plant management and production, since N

fertilizer is expensive and is susceptible to runoff or atmospheric loss (Drinkwater, 2004). To test our hypothesis, we collected live leaf material and compared it to recently senesced leaf litter to estimate resorption. We used in situ leaf litter bags to measure litter decomposition. We measured $\delta^{15}N$ values from live cranberry leaves and roots to infer ERM mediation of N uptake. Since the cranberry roots and ERM fungi colonize leaf litter, we also measured $\delta^{15}N$ values from OM layers to infer whether ERM mobilized N throughout the rooting zone.

Materials and Methods

Bed Characteristics

Data were collected from five conventionally cultivated cranberry beds on four different farms in Wood (44°30' N/ 90°0' W) and Juneau Counties (43°47' N/90°04' W) Wisconsin, USA. All beds contained the variety 'Stevens' and were relatively young plantings (9 to 12 years old). Underneath the top 15 cm layers of Wisconsin cranberry soil, which had alternating layers of organic matter and sand, was at least 30 cm of pure sand. Underneath the pure sand were substrata of either sand or peat. We selected two beds with peat substrata and three beds with sand substrata to represent both types. A grid of 35 points, 5 columns by 7 rows, was laid out in each bed, which were rectangular and between 1 to 2 hectares. Columns were typically 61 m apart, and rows were 6 m apart. All samples were collected from randomly selected points on this grid (soil cores, live plant biomass, trap litter, and leaf litter bags, and ericoid mycorrhizal fungi).

Soil N Pools

Soil N pools (ammonium, nitrate, and total dissolved nitrogen (TDN)) were measured from two (2.5 cm x 15 cm) soil cores taken from six randomly chosen points in the grid at three times throughout the 2006 growing season, May 6th, June 24th, and August 24th. The cores were transported on ice and stored at 4ºC until analyzed (within 48 hours of collection). Net N mineralization in the field was assayed with the buried bag technique (Eno, 1960). At each sampling location, a soil core was placed in a sealed polyethylene bag, re-inserted back into the hole at the same time and the same location as the soil cores. Three different sets of buried bags were incubated between five to seven weeks throughout the growing season. A 1:10 w/v soil to 1M KCl solution was used to extract N from soil cores and buried bag samples. Samples were shaken for 1 min, and extracted for 24 hrs at room temperature. Samples were then filtered with Whatman A/E 0.2 μm glass fiber filters. The extracts were frozen (-20°C) until analysis (Robertson et al., 1999). Results of extractable N from the original soil cores were subtracted from the results of the buried bags to obtain a net N mineralization rate.

Ammonium measurements were determined colorimetrically on a Labsystems Multiskan EX microplate reader (Thermo Fischer Scientific, Walthman, MA) at 650 nm absorbance using the Berthelot assay (Rhine et al., 1998). TDN measurements were made using this assay, following a persulfate digestion and a reduction with De Varda's alloy (Sims et al., 1995). We substituted phenol for 2-phenylphenol sodium salt tetrahydrate (PPS) which was a component of reagent #2 in the Berthelot assay. A precipitate consistently formed in the digested samples when PPS was used. The samples were clear when reagent #2 was made with fresh phenol, pH adjusted to 11. The absorbance values of undigested ammonium standards using phenol were identical to those using PPS (S.

Stackpoole, unpublished data). Nitrate samples were reduced to ammonium with DeVarda's alloy and then analyzed. Dissolved organic nitrogen (DON) was determined by subtracting ammonium and nitrate from the TDN values.

Live plant biomass and trap litter collections

To sample live plant material and leaf litter from OM layers, one biomass core (15 cm deep x 8 cm diameter) was taken through the plant canopy from 5 or 6 randomly chosen points on the grid in August and November 2005. Cores were stored at 4°C until processed. A sub-sample of August 2005 leaves was used in the comparison of live fresh leaves to recently senesced trap litter (see next section). Another sub-sample of August 2005 leaves was used to compare the ^{15}N natural abundance of cranberry leaves to cranberry roots. The belowground portion of the core was carefully washed to remove most of the sand. Leaves and roots were sorted from the rest of the plant material. Cranberry roots and ERM were analyzed together because it was impossible to separate the mycorrhizal hyphae from the root tissue. The November 2005 soil cores were used to compare leaf litter sub-samples from the OM layers of the soil profile. The core was split in half, and leaf litter was extracted from each of the layers with tweezers. Age of the leaf litter was roughly determined from sanding reports from the growers. It was also impossible to separate the individual components of the leaf litter, which were cranberry roots, cranberry leaf litter, and fungal hyphae. Therefore, from this point on, it will be referred to as the 'leaf litter complex', to represent all three of the components. All of the samples were dried 65°C for 48 hours, weighed, and ground in 2 ml centrifuge tubes with two stainless steel balls (0.3175 cm, McMaster Carr, Type 302, Grade 100).

Samples were analyzed for $\delta^{15}N$ and percent nitrogen (%N) on a Europa 20/20 continuous flow mass spectrometer in series with a Carlo Erba CHN analyzer (University of Wisconsin–Madison Horticulture Department). Stable isotope abundances are reported as $\delta^{15}N$ in parts per mille (‰) expressed as $(R^{sam}/R_{std} - 1)*1000$, where R is the $^{15}N/^{14}N$ ratio of either sample (sam) or the reference standard (std), which was an in-house flour standard. Precision for replicated stable isotope samples was ± 0.3 ‰.

To sample detached, senesced leaves, twelve litter traps were placed on the bed surface in May 2005 using the 35 point grid. The traps (6 cm outer diameter, 5.1 cm inner diameter) were held in place with sod stakes. Leaf litter was collected from the traps in September 2005. The majority of leaf litter fell in August and September (S. Stackpoole, personal observation). All trap litter samples were also ground in 2 ml centrifuge tubes with stainless steel ball bearings. They were analyzed for %N and %C using dry combustion on a Carlo-Erba elemental CHN analyzer (University of Wisconsin-Madison Agronomy Department). Resorption efficiency (RE) was calculated as: RE = 100 x 1-

$$\left\{ \frac{\textbf{\textit{N content in trap leaf litter}}}{\textbf{\textit{N content live leaves}}} \right\}$$

. The trap leaf litter was compared to live leaf material collected in August 2005 (described in previous section). Nitrogen concentration of the trap leaf litter and lives leaves was expressed as the N mass per leaf dry mass or milligrams N per gram of leaf litter (van Heerwaarden et al., 2003).

Surface leaf litter decomposition

One-year old leaf litter was sampled from the soil surface of four cranberry beds in April 2004. The litter was returned to the lab, sorted and cleaned, and dried at 65°C. Leaf litter bags (10 cm^2) were made of noseeum fabric (0.3 mm mesh size); roots and

hyphae were not excluded. Bags were filled with approximately 1 g dry weight of leaf litter. Thirty five leaf litter bags, containing litter originally from that bed, were placed in the grid pattern as described above. Leaf litter bags were sampled from four random locations in the grid at seven different times: November 2004; May, June, August, November 2005; and May and November 2006. Roots that had invaded the bags were separated from leaf litter; leaf litter and roots were dried and weighed separately. Sand was also carefully removed from litter. All leaf litter samples were ground in 2 ml centrifuge tubes as described above. They were analyzed for %N and %C using dry combustion on a Carlo-Erba elemental CHN analyzer (University of Wisconsin-Madison Agronomy Department). Selected samples (November 2004, November 2005, and November 2006) were analyzed for $\delta^{15}N$ values as described above.

Ericoid mycorrhizal colonization

To measure ericoid mycorrhizal fungi colonization, sub-samples of roots were taken from the top layer (1 to 2 cm) of each soil core and stored in 50% ethanol. Samples were taken in 2005 at 4 times during the growing season. Samples were cleared and stained using the methods described in (Kosola and Workmaster, 2007). Root length colonization (percent root length containing ERM structures), was determined with a dissecting microscope (40x to 90x magnification), with a line intercept method (Giovannetti and Mosse, 1980).

^{15}N Natural abundance of soil nitrogen

To measure ^{15}N natural abundance of N in the soil, resin bags were deployed for 6 weeks (June 26th – August 3rd, 2006). Bags, made of nylon stocking material, were filled

with 2.5 ml (~ ½ tsp) of anion (Dowex 1, 8 to 50 mesh size, Cl^- form) and 2.5 ml of cation resin (Dowex C-211, 16 to 50 mesh size, H^+ form). The bags were installed 15 cm below the soil surface. Three bags were installed randomly on the grid within three beds. After the bags were removed from the field, the samples from each bed were pooled to get a composite sample. They were extracted two times for 1 hour each with 2M KCL (1:10 v/v). To get a total $\delta^{15}N$ signature we used an acid trapping procedure described in (Khan et al., 1998). Ammonium and nitrate were analyzed together because ammonium values were so low in some samples as to be undetectable. We used DeVarda's alloy to reduce nitrate to ammonium, and MgO was used to free ammonium as gaseous ammonia. The N in the vapor was captured on two acidified disks (Whatman 41 6.4 mm), following a 5 to 8 hour incubation period. Samples of ammonium based fertilizer (5-10-30) were also collected from growers. Samples were analyzed for $\delta^{15}N$ using an elemental analyzer coupled with a mass spectrometer as described above.

Statistical analyses

All means and standard errors were calculated with SAS PROC MEAN (SAS 9.1 Statistical Software, Cary, NC). Data were tested for normality and homogeneity. PROC ANOVA was used to compare means of surface leaf litter $\delta^{15}N$ values from 2004, 2005 & 2006 and to compare mean C:N ratios of leaf litter in OM layers. Regression analyses were carried out in CoStat (CoHort Software, Monterey, CA).

Results

Soil and plant N pools in cultivated cranberry bogs

Extractable soil ammonium (0.97 ± 0.11 kg NH_4^+ ha^{-1}) and nitrate (0.19 ± 0.05 kg NO_3^- ha^{-1}) levels averaged over three sample periods in the 2006 growing season were low compared to DON pools (13.6 ± 1.2 kg DON ha^{-1}). The average pH was acidic, 5.3 ± 0.12. Although net N mineralization throughout the growing season was low (4 x 10^{-2} ± 4 x 10^{-3} kg ha^{-1} d^{-1}), there was no indication of net N immobilization in these soils. On average 16.5 ± 1.8 kg N ha^{-1} was removed with berry harvest, which is nearly 40% of total N added in ammonium based fertilizer annually.

Resorption and surface leaf litter decomposition

Live cranberry leaf N concentration was 0.95 ± 0.02% N. Recently senesced cranberry leaf concentration was 0.60 ± 0.03. Resorption efficiency of leaves was 35.7 ± 3.49%. The mass remaining (% of initial) of surface leaf litter decreased through time (P=0.02) (Figure 1.2a). There was also a decrease in the C:N ratio (P=0.05) (Figure 1.2b). For these two parameters, most of the changes occurred within the first year of the leaf litter bags being in the field. There was no significant difference in absolute N through time (P=0.56) (Figure 1.2c). Colonization of leaf litter by cranberry roots and ERM fungi was detected in May of 2006, which was approximately two years after the leaf litter bags were placed on the soil surface. There was no significant difference between the $\delta^{15}N$ values of the surface leaf litter from November 2004, November 2005, and November 2006 (P=0.75) (Figure 1.2d).

Ericoid mycorrhizal colonization and $\delta^{15}N$ values

Ericoid mycorrhizal colonization, measured as percent total root length, was present in all beds with an average of 42 ± 13 % of the total root length colonized by

ERM fungi. Live cranberry leaves had negative $\delta^{15}N$ values (-2.61 ± 0.27). Cranberry roots, which we could not physically separate from ERM fungi, had positive $\delta^{15}N$ values (1.04 ± 0.35). The $\delta^{15}N$ values of inorganic N extracted from soil resin were all negative (-11.36 ± 1.26) reflecting the natural abundance level of both ammonium and nitrate. Results from one ammonium based fertilizer sample (5-10-30) confirmed that its $\delta^{15}N$ value was close to atmospheric levels (0.10). The relationship between the vertical position of leaf litter layers and $\delta^{15}N$ natural abundance was significant and positive ($P = 0.004$) (Figure 1.3a). We found no significant difference between the C:N ratios of the leaf litter layers ($P = 0.25$) (Figure 1.3b).

Discussion

Comparison of cultivated cranberry farms to wildland bogs

Despite being a fertilized and managed system, soil N pools, pH, and net N mineralization rates demonstrated that cultivated cranberry farms were similar to low nutrient, acidic wildland bogs. Therefore, these data supported our hypothesis that cultivated cranberries grow in an environment where nutrient conservation strategies should be beneficial. Dissolved organic nitrogen dominated extractable soil N pools. The pH was low, and the rate of N mineralization was five to ten times slower in cranberry beds than peat bogs in North America and Europe (Bridgham et al., 1998; Verhoeven et al., 1990). The low net N mineralization rate in cranberry beds may be due to the recalcitrant leaf litter, which had a C:N ratio of around 80. The C:N ratio for peat in the bog studies varied between 26 to 50 (Verhoeven et al., 1990, Bridgham et al., 1998). Fertilizer inputs (~ 40 kg N ha^{-1}) delivered between June and July annually, provided a

flush of inorganic nutrients to the cranberry agroecosystem. Approximately 40% of the N fertilizer applied annually was removed with harvested berries. The rest was stored in plant and soil pools, or lost to the environment.

Moderate to high resorption rates and slow litter decomposition

The N concentration of senesced leaves (0.60) indicated that cranberry plants went through complete resorption (Killingbeck 1996). The resorption values (between 40% to 50%) determined from van Heerwaarden's (van Heerwaarden et al., 2003) fertilized native bog study using the evergreen ericaceous species Empetrum hermaphroditum, were comparable our cranberry resorption estimate (36.5%), although ours was lower. The slight decrease in resorption efficiency, despite higher fertilization rates, indicates that nutrient status is not the only factor that can influence resorption efficiencies. Low water availability (del Arco et al., 1991) and shade (Chapin and Moilanen, 1991) can also reduce resorption efficiency below potential rates. To our knowledge, the influence of mycorrhizal status of plants on resorption rates has not yet been investigated (Aerts 1996; Killingbeck 1998). Since fungi are able to access nutrients that are sometimes unavailable to the plant, when considering the influence of soil fertility on resorption efficiencies, it may be useful to also consider DON soil pools, organic matter accumulation, and the mycorrhizal status of plants.

The decomposition rates of cranberry more closely resembled ericaceous tundra plants grown in unfertilized conditions, rather than an agronomic crop such as corn, where sizeable additions of N from plant residue (40 to 80 kg N ha^{-1}) are added to the

agroecosystem annually. For example, decomposition rates for cranberry leaf litter were higher (57 % initial mass remained) than the evergreen ericaceous species *Vaccinium vitis-idaea*, (roughly 80% initial mass remained) in an unfertilized incubation experiment at 4ºC and 10ºC (Hobbie, 1996). This was presumably because there were higher initial N values in cranberry leaves (0.95% vs. 0.70%) and higher surface temperatures in Wisconsin cranberry bogs compared to the temperatures of the incubation experiment. However, compared to a highly managed agronomic crop such as corn, cranberry decomposition was much slower. After two years in the field, only 20% of corn leaves in surface leaf litter bags remained (Burgess et al., 2002).

There was a slight increase in absolute N values during the first half of the study, followed by a slight decrease. However, there was no statistically significant net import or export of N from the surface leaf litter. This suggests that the decrease in the C:N ratio of surface leaf litter through time was due changes in carbon, most likely carbon use by microbial communities (Lindahl et al., 2007). C:N ratios of less than 25 (dry weight plant material) have been set as thresholds for net N mineralization from decomposing litter (Berg and Mc Claugherty, 2003). Using this value as an indicator, after 2.5 years in the field, it was likely that N was still being immobilized by microbes decomposing the surface leaf litter.

$\delta^{15}N$ values indicate ERM fungi access N from rooting zone

Our data supported the hypothesis that ERM fungi mediated N uptake in cultivated cranberry beds, since cranberry leaves were depleted in ^{15}N and the cranberry roots and ERM were enriched. We were also able to link the plant isotope data with the

leaf litter complex isotope data to trace the $\delta^{15}N$ values through various stages of cranberry leaves' life cycle (Figure 4). We began by focusing on the ^{15}N depleted live cranberry leaves (Figure 4a). During the process of resorption, there was no discrimination against ^{15}N (Kolb and Evans, 2002), so when the ^{15}N depleted cranberry leaves senesced and fell (Figure 4b), a layer of depleted leaf litter formed on the surface of the cranberry bed. Root colonization of the leaf litter was detected at the beginning of the second year of the study, but there were no changes in the surface leaf litter $\delta^{15}N$ values within the first 2.5 years. However, we found higher $\delta^{15}N$ values in the layer directly beneath the soil surface, and we used sanding dates from the growers to estimate that ERM fungi began mobilizing N 2.5 to 6 years after the leaf litter initially fell. We were not able to determine from the leaf litter isotope values the source of N. Most likely it was fertilizer N, but there is also a possibility that organic N was mobilized directly from the leaf litter. A tracer study, which included ^{15}N enriched leaf litter, would need to be implemented to distinguish the contribution of N from these two sources.

Our mycorrhizal colonization rates were similar to those found by Kosola and Workmaster (2007), and they also measured higher mycorrhizal colonization rates in the shallowest OM layers. We believe that there was more fungal activity near the surface, and with time the leaf litter complex was buried by additional applications of sand every 3 to 5 years (Figure 4d), and that is where we found the highest $\delta^{15}N$ values. The deepest soil layers we examined were 9 to 12 years old. This isotope pattern could also be explained by plant uptake of N that is depleted in ^{15}N relative to soil total N, followed by deposition of isotopically light N on soil surface (Nadelhoffer and Fry 1994). These factors may also contribute to the ^{15}N natural abundance patterns in the cranberry

agroecosystem, but our data also strongly suggest that ERM fungi influenced the distinct $\delta^{15}N$ values of cranberry roots and leaves, as well as the $\delta^{15}N$ values with increasing depth in the OM layers.

The C:N ratios of the leaf litter complex were not what we expected and did not contribute much to our overall understanding of decomposition processes in the organic matter layers. Other field-based studies found an increase in C:N ratios in leaf litter that was located in deeper layers of the soil profile (Kosola and Workmaster, 2007; Lindahl et al., 2007). There was a slight decrease in C:N ratios between Layer 1 and Layer 2, followed by a slight increase between Layer 2 and Layer 3. The trend was not statistically significant, and therefore we were unable to infer net immobilization or net mineralization of N from these older leaf litter layers. This could be due to the shorter time frame of our cores, which were 11 years old. In Lindahl's (2007) study the cores were 45 years old, and the cores were 15 years old in Kosola's (2007) study.

$\delta^{15}N$ of inputs and microbial processing of OM

We also measured the ^{15}N natural abundance of the main inputs to the cranberry agroecosystem, since it has been documented that $\delta^{15}N$ values of N sources can also influence $\delta^{15}N$ values of plant leaves (Michelsen et al., 1998; Schulze et al., 1994). Ammonium based fertilizer, applied in June and July accounted for 60 to 70% of N inputs to the system (S. Stackpoole unpublished data), and the $\delta^{15}N$ value for that N source was close to zero. The $\delta^{15}N$ value of the inorganic N (both ammonium and nitrate) from soil resin extracts was negative (-11). This value probably reflects an integration of multiple N transformation processes, which may include nitrification and possibly

dissimilatory nitrate reduction to ammonium (DNRA) (Ostrom et al., 2002). The low ^{15}N signature of the inorganic soil solution relative to the plant indicates that N mineralization from litter contributed small amounts of N to the cycle. Therefore, the negative cranberry leaf $\delta^{15}N$ was a result of total N inputs including fertilizer N (zero $\delta^{15}N$), soil N pools (strong negative $\delta^{15}N$), and possibly leaf litter N (negative $\delta^{15}N$). Cranberry plants may draw upon the latter two nutrient sources later in the growing season when fertilizer applications have ceased at the end of July and fruit growth is at a maximum in August (Hart et al, 2000). The resin bags had a similar mean value for ammonium (1.87 mg l^{-1} ± 1.46) as the soil extracts (1.08 mg l^{-1} ± 0.04), but the resin bags had a higher mean value for nitrate (2.17 mg l^{-1} ±1.09) than the soil extracts (0.16 mg l^{-1} ± 0.04). We assumed this is because the resin tightly bound the nitrate, while nitrate was more prone to leaching because of the low cation exchange capacity of the sandy soil.

Conclusions

We found that even with N additions through fertilizer, cranberries exhibited traits of plants growing in nutrient limited conditions, including litter with high C:N ratios, and slow decomposition. Additionally, the distinct soil OM layers in cultivated cranberry beds provided a unique system to study ERM effects on N cycling. Using ^{15}N natural abundance measurements, we were able to provide evidence that ERM fungi mobilize N from the rooting zone. Until now, the role of ERM fungi in tightly regulating N cycling was not recognized, and most cranberry growers did not account for ERM influences on N uptake. This may have important environmental and management implications, since inorganic N fertilization is susceptible to runoff and atmospheric loss. Future work

should quantify the N flux through the ERM pathway to determine if and how much organic N is mobilized by ERM fungi from organic matter relative to fertilizer inputs.

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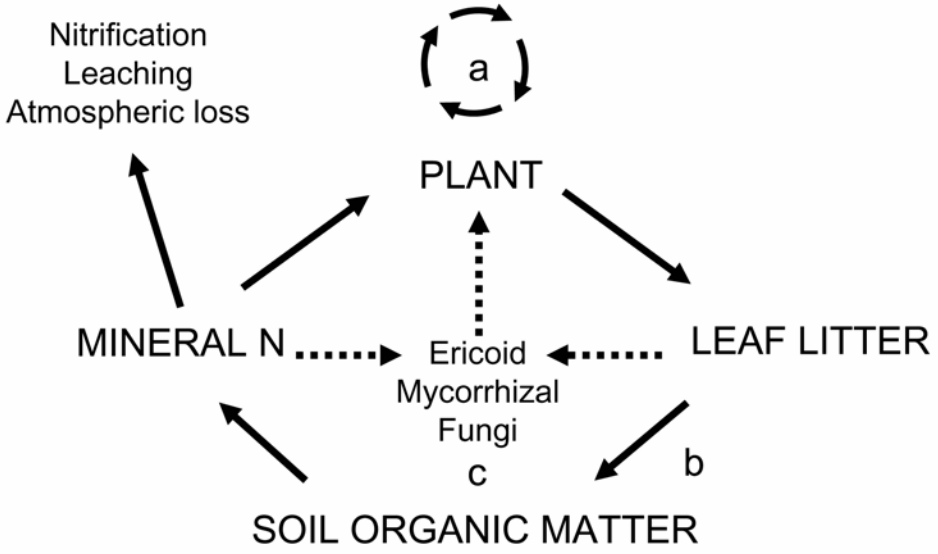

Figure 1.1. Important N pathways in the cranberry agroecosystem. Solid arrows indicate the standard pathway of N cycling, which is prone to N loss from the system. (a) Nitrogen is resorbed from leaves prior to senescence and leaf fall. (b) Fallen leaf litter decomposes, becomes part of the soil organic matter, and then it is mineralized to ammonium, which can be taken up by the plants. (c) The dotted arrows indicate the pathway where N from fertilizer, mineralization, or even organic N from leaf litter may be taken up directly by plants via their ERM symbiosis.

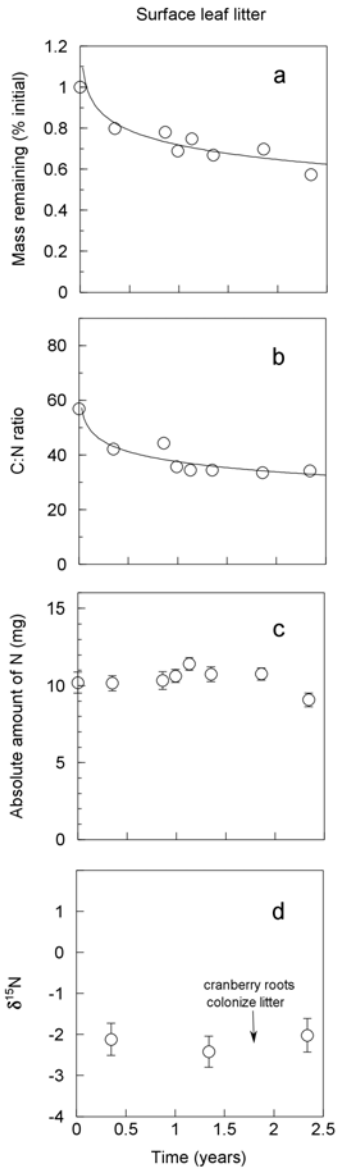

Figure 1.2 Decomposition of leaf litter bags placed on the soil surface, means ± 1 S.E. Standard errors are shown where larger than size of symbol. (a) Mass remaining ($y=0.72-0.11*\ln(x)$; $R^2=0.7$; n=25) (b) C:N ratio ($y=37.54-5.39*\ln(x)$; $R^2=0.58$; n=25). (c) There was no significant change in absolute amount of N through time. For $\delta^{15}N$ values only samples from three collection times were analyzed and presented (November 2004, 2005, & 2006), and there was no significant difference among the means.

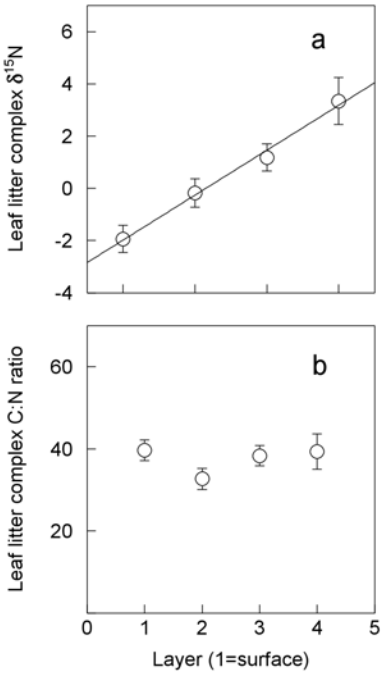

Figure 1.3 Changes in $\delta^{15}N$ values and leaf litter chemistry with vertical position of the leaf litter layers. Means ± SE are plotted for three beds. (a) δ15N of leaf litter and ERM (y=-3.60 + 1.72* x, R^2=0.99, n=9) (b) C:N ratio of leaf litter and ERM (y=36.35+0.469*x, R^2=0.03, n=9).

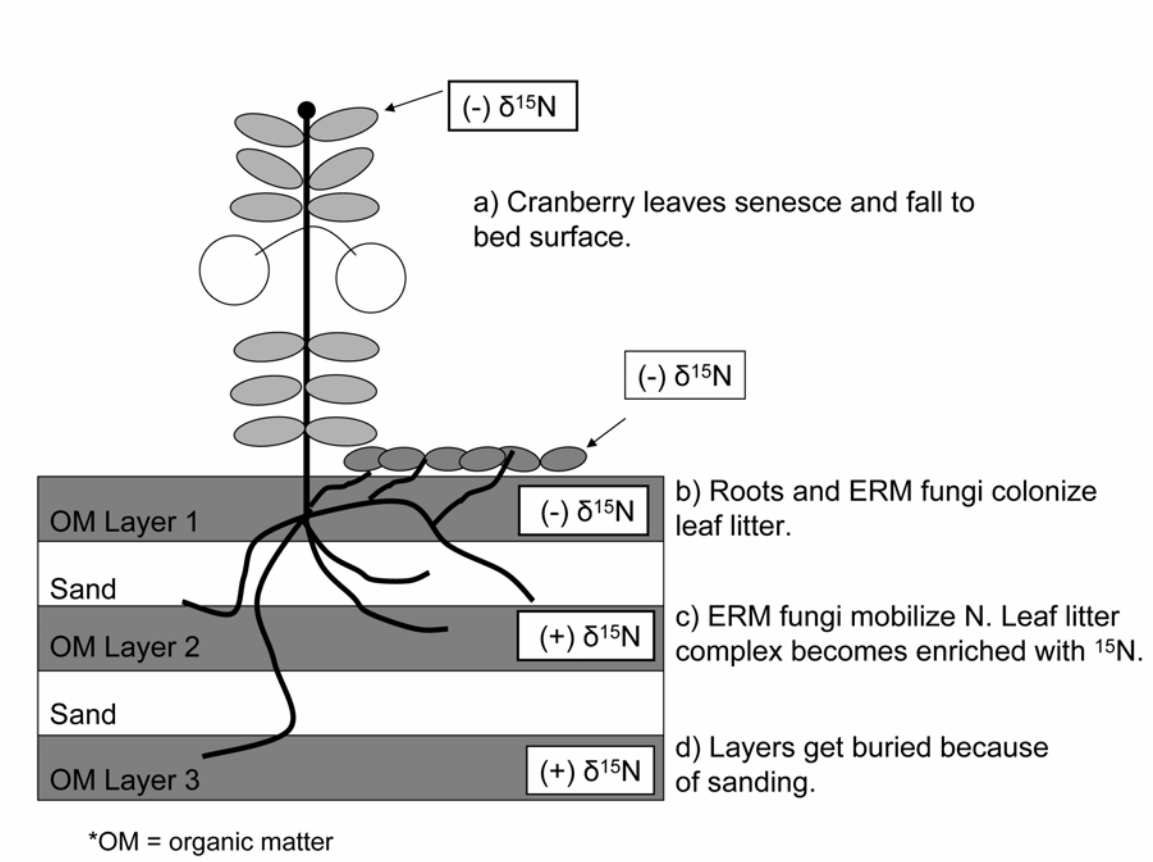

Figure 1.4 Tracking the changes in $\delta^{15}N$ values in cranberry leaves' life cycle. The ^{15}N depleted cranberry leaves senesced and fell (a) which produced a layer of depleted leaf litter on the surface of the cranberry bed. Eventually cranberry roots and ERM began to colonize the leaf litter (b). The ERM fungi mobilized and transferred N to the plant, and the heavier isotope remained in the fungal hyphae. Since the ERM was so closely associated with the leaf litter tissue, the ^{15}N level of this material was also positive (c). With time, these leaf litter layers got buried because cranberry growers applied a layer of sand to the bed surface every 3 to 5 years (d).

Chapter 2. Characterizing N cycling to assess the sustainability of cranberry

Abstract

Net accumulation of nutrients, use of organic N compounds, and the influence of ericoid mycorrhizal fungi (ERM) on nutrient cycling have been demonstrated in pristine systems like the arctic tundra and the boreal forest, but they have not been extensively investigated in agricultural systems in relation to sustainability. The objective of this study was to measure net accumulation of agroecosystem N, create an N budget in order to determine the relative importance of organic and inorganic N as nutrient sources, and highlight the potential role that ericoid mycorrhizal fungi (ERM) may play in N cycling for the cranberry agroecosystem. We measured ERM colonization, and the main N inputs, outputs, and pools in cultivated cranberry beds. Overall, net N inputs exceeded net N outputs, indicating that cranberry beds were a sink for N. Inorganic N fertilizer was the dominant form of N added to the system (43 kg N ha^{-1} yr^{-1}), but dissolved organic N dominated soil N pools, floodwater, and irrigation water. The berry harvest removed ~10% of biomass N each year, but a similar amount of N was recycled back into the system through stem- and litterfall. We inferred from our N budget that organic N sources and ericoid mycorrhizal fungi play an important role in N cycling and cranberry nutrition in fertilized systems. This result does not support the idea that DON is only important in low-fertility ecosystems and has important ramifications for management,

since growers and current management plans focus in labile N inputs and overlook the potential for nutrient recycling within the system.

Key words: ericoid mycorrhizal fungi, dissolved organic nitrogen, *Vaccinium macrocarpon*

Introduction

The sustainability of an agroecosystem is closely linked to its nutrient cycling. One inherently sustainable aspect of some agroecosystems is the tendency for nutrients to accumulate, despite some losses from erosion, leaching, or volatilization (Woodmansee, 1984). A second measure of agroecosystem sustainability is its ability to naturally recycle and draw upon nutrients from plant litter and soil organic matter rather than external inputs, such as fertilizer. A third gauge is the composition and functioning of the soil microbial community such as abundant mycorrhizal fungi that link biotic and geochemical components of the ecosystem to enhance nutrient uptake by crops (Jeffries and Barea, 1994).

Based on work in temperate broadleaf forests and coastal sand dunes, the nutrient retention hypothesis posited that the degree to which nutrients, specifically N, leak from an ecosystem is related to the age of that system or its time since disturbance. As ecosystem succession progresses, the biomass and soil organic matter are growing storage pools for nutrients entering the system (Vitousek and Reiners, 1975). A corollary of this hypothesis is that ecosystems that are not managed well will be leaky with respect to inputs of a limiting nutrient because plants in a constant state of recovery cannot keep pace with these inputs. Exogenous nutrients that do not pass through the ecosystem can

accumulate in biomass and soil organic matter and then be recycled for plant uptake via mineralization and mycorrhizal activity. Some mycorrhizae, especially those in nutrient-poor systems such as heathlands or the arctic tundra, have saprotrophic capabilities that facilitate nutrient mobilization from plant residues (Read and Perez-Moreno, 2003).

Examination of current cranberry production lends itself to a sustainability study since native cranberries are grown in environmentally sensitive areas such as wetlands and uplands adjacent to rivers and streams, and N leaking from these agricultural areas could impact downstream aquatic systems. Cranberry beds are agroecosystems that exhibit many of the same characteristics of nutrient-poor wildland systems (Schimel and Bennett, 2004) i.e. high plant biomass C:N, relatively large pools of dissolved organic N (DON), and the presence of ericoid mycorrhizal (ERM) fungi (Stackpoole et al. 2008). However, the existing work has not shown the extent to which cranberry plants access labile N applied annually as ammonium or recyling organic N via the ERM symbiosis.

Demonstrating that organic N pools are important to crop growth despite fertilizer N inputs is important from a management perspective, but would also shed light on the position of this high-yielding agroecosystem along the resource gradient proposed by Schimel and Bennett (2004). This conceptual model placed agricultural systems at one end and wildland systems at the other, based upon the supply of inorganic N. In the cranberry agroecosystem, inorganic N should be available from fertilizer inputs, but we also wanted to find evidence that large organic N pools were also accessed by plants. Understanding that cranberry plants use organic N pools may allow farmers to apply less

fertilizer and thereby reduce N losses to groundwater, the atmosphere, and downstream aquatic ecosystems.

The objective of this paper was to use a N budget of the cranberry agroecosystem to infer overall accumulation of N in the system and determine the importance of organic N, such as leaf litter and soil solution DON, as N sources relative to fertilizer inputs. Given our current understanding about the links between cranberry plants, ericoid mycorrhizal fungi, and organic N sources, we hypothesized that leaf litter and DON sources represented significant N pools in the cranberry N budget. Since ERM fungi are important to increased absorption and recyling of N, we documented the colonization rates of cranberry plants in the cranberry agroecosystem. We also measured total plant biomass and soil extracts as residual N stocks. Since we selected relatively young cranberry beds, we predicted that biomass would accumulate and therefore that inputs would be greater than outputs and the difference should be about equal to the amount of N accumulated in biomass annually. Furthermore, we measured N entering and leaving the system via water from precipitation, irrigation, and floodwater. We inferred contributions of N from capillary rise and loss of N via leaching from a water budget and we incorporated gaseous N emission estimates from the literature. We hypothesized that the inorganic N inputs from fertilizer, irrigation, and precipitation alone would not be great enough to account for the amount of N that accumulated in the cranberry biomass annually. Therefore, organic N sources, which had not previously been accounted for would also need to be included in the overall N budget.

Methods

Cranberry bed characteristics and sampling

We collected data from five conventionally cultivated cranberry beds on four farms in Wood (44°30' N, 90°0' W) and Juneau Counties (43°47' N, 90°04' W) Wisconsin, USA. All beds contained the variety 'Stevens' and were relatively young plantings (9 to 12 years old). It takes three to four years to establish new cranberry beds, and some can remain in production for up to forty years (Eck, 1990; Kosola and Workmaster, 2007). The surface (5 to 10 cm) of cranberry soil at our research sites was characterized by alternating layers of organic matter and sand. This pattern occurs because the growers apply 1 to 2.5 cm of sand to the beds every 3 to 5 years in the winter, which is typical in Wisconsin cranberry production. Below the alternating layers of organic matter (leaf and stem material) and sand, was at least 30 cm of pure sand. Below this were substrata of either sand or peat. We selected two beds with peat substrata and three beds with sand substrata to represent both types. Beds were rectangular, 1 to 2 ha in area, and were surrounded by a drainage ditch (0.3 m wide, 0.15 to 0.61 m deep). A grid of 35 points (5 columns by 7 rows) was established in each bed. Columns were 61 m apart, and rows were 6 m apart. All samples were collected from randomly selected points on this grid.

Ericoid mycorrhizal colonization

To sample ericoid mycorrhizal colonization we removed one core (15 cm deep × 8 cm diameter) from 5 or 6 randomly chosen points on the grid described above at 4 times during the 2005 growing season (May, June, August, and November). Cores were

stored at 4°C until processed. Subsamples of roots were taken from the surface layer (1 to 2 cm) of each soil core and stored in 50% ethanol. Samples were cleared and stained using the methods described in Kosola and Workmaster (2007). Root length colonization (percent root length containing ERM structures) was determined with the line intercept method using a dissecting microscope (40× to 90× magnification) (Giovannetti and Mosse, 1980).

Plant biomass and litter fall

Live biomass stocks included live leaves, aboveground stems, berries, roots and belowground stems. Aboveground necromass, such as dead stems, was also measured. To sample the plant material one core (15 cm deep × 8 cm diameter) was taken through the plant canopy from 5 or 6 randomly chosen points on the grid in late August or early September of 2004, 2005, and 2006. Cores were stored at 4°C until processed. Aboveground leaves, stems, and berries were separated from the belowground portion of the core. The belowground portion was carefully washed to remove sand, but we also corrected for sand by taking the ash-free dry weights of the samples. Belowground biomass contained primarily cranberry plant roots, but sometimes it was difficult to distinguish between a stem and a root. In addition, it was impossible to remove the fungal material from the roots, so fungal biomass was also included in the belowground measurements. All of the samples were dried at 65 °C for 48 hours, weighed, and ground in 2 ml centrifuge tubes with two stainless steel balls (0.3175 cm, McMaster Carr, Type 302, Grade 100). The N and carbon (C) were determined for these samples using dry

combustion on a Carlo-Erba elemental analyzer (CE Elantech EA1112, Lakewood, N.J.). Berry yield was provided by grower records.

To sample detached, senesced leaves, twelve litter traps were placed on the bed surface in May 2005 using the 35 point grid. The traps (6 cm outer diameter, 5.1 cm inner diameter) were held in place with sod stakes. Leaf litter was collected from the traps in November 2005 and 2006. The majority of leaf litter fell in August and September. All of the biomass and trap litter samples were dried, weighed and analyzed as stated previously.

Soil solution pools, fluxes, and soil organic matter

Soil solution N pools (NH_4^+, NO_3^-, and total dissolved N [TDN]) were measured from one core (15 cm deep × 8 cm diameter) taken from five randomly chosen points on the grid at four times (May, June, August, and November) in 2005. The surface 0 to 5 cm of the core, which included the alternating layers of litter and sand, was analyzed separately from the lower 5 to 15 cm of the core, which was pure sand. The practice of taking large diameter soil samples and analyzing the two layers separately was discontinued in 2006 because the larger soil core damaged the cranberry vines.. Therefore in 2006, two soil cores (2.5 cm × 15 cm) were taken from six randomly chosen points in the grid at three times (May, June, and August) throughout the growing season. The cores were transported on ice and stored at 4ºC until analyzed (within 48 h of collection). Net N mineralization in the field was assayed with the buried bag technique (Eno, 1960). At each sampling location, a soil core (2.5 cm × 15 cm) was placed in a sealed polyethylene

bag and re-inserted into the hole at the same time and the same location as the soil cores. The buried bags remained in the field for five to seven weeks.

A 1:10 w/v soil to 1M KCl solution was used to extract N from soil cores and buried bag samples. Samples were shaken for 1 min, and extracted for 24 h at room temperature. Samples were then filtered with Whatman A/E 0.2 μm glass fiber filters. The extracts were frozen (-20°C) until analysis (Robertson et al., 1999). Results of extractable N from the original soil cores were subtracted from the results of the buried bags to obtain a net N mineralization rate.

Ammonium measurements were determined colorimetrically on a Labsystems Multiskan EX microplate reader (Thermo Fischer Scientific, Walthman, MA) at 650 nm absorbance using the Berthelot assay (Rhine et al., 1998). Total dissolved N measurements were made using this assay, following a persulfate digestion and a reduction with De Varda's alloy (Sims et al., 1995). We substituted phenol for 2-phenylphenol sodium salt tetrahydrate (PPS) which was a component of reagent #2 in the Berthelot assay. A precipitate consistently formed in the digested samples when PPS was used. The samples were clear when reagent #2 was made with fresh phenol, pH adjusted to 11. The absorbance values of undigested ammonium standards using phenol were identical to those using PPS (S. Stackpoole, unpublished data). Nitrate samples were reduced to ammonium with DeVarda's alloy and then analyzed. Dissolved organic N was determined by subtracting NH_4^+ and NO_3^- from the TDN values.

Three cores were taken from five beds in November 2005 to measure bulk density. Each soil core had three to four distinct layers of leaf litter, and since the layers

ranged in age and stage of decomposition, we averaged the N and C concentration across all of the layers. The bulk density measurement was multiplied by the average N and C concentration of the organic matter within the core to derive an estimate of soil N and C mass within the surface 15 cm.

Water sampling

In addition to soil and plant pools, we also measured N in various water sources of the cranberry agroecosystem. Wet deposition inorganic N content was provided by the National Atmospheric Deposition Program/National Trends Network (NADP/NTN, http://nadp.sws.uiuc.edu/) (Holland et al., 2005; Lamb and Bowersox, 2000). At the NADP/NTN Portage County, Lake Dubay site (44º7' N 89º8' W) precipitation accumulated in buckets that were triggered to open at the onset of rain. Samples from the buckets were collected weekly. We averaged the wet deposition data annually.

In 2005 automated samplers (Teledyne ISCO, Lincoln, Nebraska) drew samples of actual influent and effluent of floodwater from two different beds (Beds 4 and 5). Floodwater, irrigation and groundwater sampling focused on three beds (Beds 1, 4, and 5). Two grab samples of standing floodwater were taken during the period of spring or fall harvest flood in 2006. In 2006, grab samples were taken from irrigation water reservoirs 2 to 3 times during the growing season. Groundwater samples were taken from minipiezometers in 2006. Three minipiezometers were installed along a transect which started approximately 2 meters from the edge of the bed at 10 m intervals in Beds 1, 4, and 5. Minipiezometers were constructed from polyethylene tubing (0.43 cm i.d., 0.63 cm o.d.). Well screens, placed on one end of the minipiezometer, had 8 to 10 lines of

perforations 2.5 cm long, which were created in four to five passes through a sewing machine. Pipetter tips (101-1000 uL size, Fisherbrand specialty #21-375E) were attached on top of the screen. A retractable tempered stainless steel insertion rod was used to position the well screens at depths about 150 cm. Groundwater samples were taken using a peristaltic pump attached directly to the minipiezometer (Browne and Guldan, 2005). Samples were collected during the growing season May through August. Samples were field filtered through 1.5 μm glass microfiber filters, stored on ice for transport, and frozen until analysis for total N, NH_4^+, and NO_3^- as described previously. Additionally, water table depth was also measured with a probe that was inserted into the minipiezometer that detected the height of the water within the tube.

Nitrogen concentration of water samples was determined by the same protocol used for soil extracts and concentration values were multiplied by average volume of water to calculate N on a mass basis. The volume of precipitation was obtained by rain gauges placed in Beds 1 and 2. The grower at this location recorded and reported the amount of water accumulated in rain gauges on a daily basis. Volume of water from irrigation was given by individual growers and was based on the amount of time pump was turned on and amount of water delivered by the sprinklers per hour. Height of floodwater was generally set at 1 m, so volume floodwater was calculated based on bed area.

The water budget contained the following components and three main assumptions:

Δ SM = (P + I + FI + C) – (ET + FO + L + S) (See Table 2.1 for an explanation of the budget terms). The first assumption of this budget was that there was negligible surface runoff (S) during the growing season (Hillel, 2004). We felt confident in this assumption because cranberry beds are precision leveled at time of construction and the subsurface sand was highly permeable. The second assumption was the amount of incoming floodwater (FI) roughly equaled the amount of outgoing floodwater (FO), which preliminary data from 2005 supported. The third assumption was that we could not determine the absolute volume of water inputs from capillary rise (C) and water losses from leaching (L). Other studies have used lysimeters to measure leaching from the rooting zone (Baker and Timmons, 1994; Brye et al., 2001). However, the high water tables (<0.5 m) in cultivated cranberry beds made lysimeter installation problematical (Bland et al., 1996). Therefore, we simplified our water budget equation to: Δ SM = (P + I) – (ET), and the net volume and direction of water across the rooting zone were inferred from the outcome of a growing season (May through October) root zone water balance (Hillel, 2004; Loheide, 2008). We were able to determine the total water volume crossing the rooting zone, but we could not determine the vertical direction of the flow. We did measure water table depth in three beds to determine if the water table was likely to be high enough to allow capillary rise from groundwater to contribute inputs to the rooting zone.

County level daily potential evapotranspiration data was provided by Wisconsin-Minnesota Cooperative Extension Agricultural Page (http://www.soils.wisc.edu/wimnext/index.html). The method used for estimating potential evapotranspiration was on a formulation developed by Priestly and Taylor,

where ETp= 1.26(Rn – G)S/S+γ), where Rn is the net radiation, G is the soil heat flux, S is the slope of the saturation vapor pressure at the temperature of the air (hPa K^{-1}), and finally γ is the psychrometric constant (hPa K^{-1}) (Diak et al., 1998) To apply these values to the cranberry crop, we divided ET estimates from the database by 1.26 to get to the equilibrium evapotranspiration rate. We then multiplied that value by 1.1, because evapotranspiration rates in cranberry are about 110% of the equilibrium evaporation rate (Bland et al., 1996). These values were then summed over the growing season.

Nitrogen budget

The overall equation for the N budget was: $Inputs_N – Outputs_N = Residual_N$. The terms of an N and water balance are listed in Table 2.1. The time frame of the budget was the growing season, defined as May 1 through October 1, 2006, so a spring flood (early May) and a harvest flood (early October) were also included. We also defined a spatial framework for the budget that extended 15 cm above the soil surface to 15 cm below the soil surface to include the plant canopy through to the rooting zone. Plant and soil pools within these boundaries were defined as N pools internal to the system and any turnover of nutrients within these boundaries was an internal flux. Any movement of N across these boundaries, such as vertical water movement from the rooting zone to groundwater or irrigation water coming in from above the plant canopy, were considered outputs or inputs to the system. Horizontal water flows in the ditches or in the bed were not measured in this study. Emission, denitrification, and leaf litter harvest N values were obtained from the literature.

Data analysis

All means and standard errors were calculated with SAS PROC MEAN (SAS Statistical Software, Cary, NC). For regression analysis, PROG REG was used to compare fertilizer application rate to yield and to estimate the rate of increase of N and C in plant biomass on a kg ha^{-1} basis.

Results

The estimated rate of N accumulation in biomass every year was 64.40 ± 14.44 kg N ha^{-1} ($p<0.001$, $y = 64.40 \times year - 95.33$). About 75% of the total vegetative biomass in cranberry beds was belowground (Table 2.2). Between 5 and 10% of the N stored in total biomass was removed from the system in harvested berries each year. The estimated C accumulation in biomass each year was 1872.17 ± 694.75 kg C ha^{-1} ($p<0.01$, $y = 1872.17 \times year + 7533.54$). Sixty percent of the carbon in vegetative biomass was belowground. Between 10 and 13% of the C stored in total biomass was removed from the system in harvested berries each year. Approximately 10% of the total N and about 20% of total C in vegetative biomass was added to the bed surface through fallen leaves and stems annually (Table 2.2). Fallen leaves and stems had C:N ratios of 87.2 ± 2.3 and 81.9 ± 2.1, respectively.

Dissolved organic N dominated the extractable soil N pools relative to NH_4^+ and NO_3^- (Figure 2.1a). Nitrogen pools in the surface 15-cm were similar to those deeper in the soil profile (Figure 2.1 a & b) and were similar in 2005 and 2006 (Figure 2.1 b. & c). The bulk density of cranberry soils ranged from 1.20 to 1.29 g cm^{-3}. Carbon and N in the surface 15-cm were 49.4 Mg C ha^{-1} and 1.4 Mg N ha^{-1}, respectively. Ericoid mycorrhizal

colonization was detected in all beds in all samples. Colonization measured as percent total root length, was present in all beds with an average of 42 ± 13 % of the total root length colonized.

We estimated that on an annual basis 1483 L of water entered the cranberry beds through floodwater, irrigation, and precipitation. Nearly, 20 kg N ha^{-1} yr^{-1} was added to the cranberry agroecosystem through water inputs (Table 2.3). Every aqueous N component of the cranberry bed contained DON (Table 2.3), indicating that DON was a component of water fluxes as well as extractable soil N. The N concentrations of the water sources were similar, but the total amount of N added to the system was influenced by the volume of water. In particular, the amount of irrigation varied greatly among the three beds (Table 2.4).

Our water budget indicated that growing season precipitation and irrigation exceeded evapotranspiration rates, so the average amount of water that crossed the rooting zone was positive, around 12.2 kg N ha^{-1}. This value we kept for our leaching estimate (Table 2.3). However, we were more conservative with our estimated amount of N contributed from capillary rise. Estimated capillary rise for sandy soils in our study beds was between 12 and 35 cm (Hillel, 2004), so from our water table depth measurements, capillary rise could have contributed to the rooting zone periodically during the growing season for beds 1 and 4, but not for Bed 5. The water table depths in Bed 5 were consistently too low throughout the growing season for capillary rise to be great enough to contribute N from groundwater to the rooting zone. For Beds 1 and 4, capillary rise was not considered an input during periods where the water table was high, because it was internal to the system. Since we didn't measure water tables depths on a

daily basis, we determined that the maximum average volume of water from capillary rise had to be <12.2 kg N ha^{-1}.

The difference between N outputs and inputs was defined as the residual N, and was positive for the cranberry agroecosystem (Figure 2.2). To complete the balance estimates for N loss through emission (Hemond, 1983), denitrification (Aerts et al., 1999); Bowden, 1987; Urban et al., 1988) and leaf harvest (Hart et al., 2000) were taken from the literature (Figure 2.2) Twenty six percent of total N inputs were from water sources, while the balance was from fertilizer. Nearly half of the total annual inputs were removed with harvest leaves and berries. Just over half of annual inputs returned to the system via litterfall and stemfall.

Discussion

Fertilizer was the largest N input to the agroecosystem, but previous studies of other horticultural crops have indicated relatively low percentages of N fertilizer are taken up by woody perennial crops. Raspberries, with a perennial rootstock and a biennial top, derived only 13 to 36% of N from fertilizer (Rempel et al., 2004). In a study with perennial pecan tress, 28% of ^{15}N labeled fertilizer was found in the plant, 24% in the soil, and 48% was lost to the environment (Rey et al., 2006). These values are much lower than those reported for agronomic crops such as corn, which has a whole plant fertilizer recovery of about 54% (Bundy and Andraski, 2005). Although cranberry plants may not be assimilating fertilizer N, it is possible that a portion of the fertilizer is being assimilated by soil microbes, which in turn are helping to decompose organic matter in the agroecosystem.

Cranberry growers have found that it takes three years before berry yields begin to suffer from lack of fertilizer (Hart et al. 2000), and this may be due to the plants' ability to draw nutrients from root reserves (Stieber and Peterson, 1987). For perennial fruit crops, N stored from the previous year may be used to support new growth, even when fertilizer N was available (Smith et al., 2007). In rabbiteye blueberry (*Vaccinium ashei* Read), storage tissues provided 90% of the total N to flowers and young fruit during anthesis (Birkhold and Darnell, 1993). Hart et al. (2000) found that only 5 to 10% of N comes from fertilizer for cranberries, and that the remaining 90% or about 45 kg N ha^{-1} comes from soil organic matter and old plant tissue.

Large nitrogen and carbon pools in biomass and organic matter

Nitrogen accumulated in the cranberry beds we studied and was accompanied by C accumulation, which likely provided the additional storage capacity for N (Janzen et al., 2003). Our estimates of soil organic matter C and N pools, in the surface to a depth of 15 cm, exceeded vegetative biomass pools. Our data indicated that there is potential to store large reserves of C and N in soil organic matter, which is likely driven by slow decomposition of the leaf and stem material (Stackpoole et al., 2008). There was a decrease in belowground biomass C from 2004 to 2005, which may have stemmed from the application by farmers of an herbicide that reduces root growth (Sandler et al., 2004). Overall, these data provide evidence that this perennial cropping system can have a positive effect on nutrient retention through storage of both C and N in biomass and organic material. The net accumulation of nutrients in biomass can have important

environmental consequences in the form of reduced downstream nutrient runoff (Gregory et al., 2004; Janzen et al., 2003; Vitousek and Reiners, 1975; Woodmansee, 1984) .

Organic N pools were large relative to inorganic N pools and fluxes

Dissolved organic N dominated extractable soil N pools during the growing season. In other low N systems, such as taiga, DON pools can be six times the inorganic N pool (Jones and Kielland, 2002). In other perennial agricultural systems, DON was a negligible component of the total N pool (Christou et al., 2006; Christou et al., 2005). In our cranberry systems, DON was also found in floodwater, which may come from: 1) leaching and microbial decay of humic materials, 2) root and microbial exudates, and/or 3) turnover of roots (Kalbitz et al., 2000). Irrigation water, which had the same source as floodwater, had a major DON component too. The inorganic N in the irrigation water was most likely the result of microbial transformation of DON during the warmer times of the growing season or nitrate contamination from surrounding agricultural communities.

Our budget results indicated that DON could be an additional source of N for the plant since plant N exceeded available N. Similar reasoning has been used to infer DON use by plants in arctic tundra (Kaye and Hart, 1997; Kielland, 1994). The budget was a useful tool because it is difficult to infer plant use of DON by measuring pool sizes alone. The DON pools were large relative to the inorganic N pools, but we do not know the flux of N through the DON pool. Since the budget portion of our study suggested that DON was being used, the rate at which DON was taken up by plants was probably close to the rate at which DON was added to the extractable soil N pools. This large pool of DON

could be in the form of labile amino acids, or it could be comprised of more complex tannin-bound proteins. Either of these forms would be accessible to ERM-colonized plants. It is also possible that a portion of the DON pool was mineralized by soil microbes. We found positive net N mineralization rates within the rooting zone, but they were relatively small.

Studies of peatland systems have shown the importance of nutrient recycling via mineralization (Bowden, 1987; Bridgham et al., 1998; Hemond, 1983). Five to 10% of the plant biomass N was removed with the berry harvest each year, but approximately 10% of this N was returned to the system via leaf and stem litterfall. The litter had very high C:N ratios and very slow decomposition rates (Stackpoole et al., 2008), but it has been demonstrated that ERM fungi can act like saprophytic fungi and can facilitate mobilization of nutrients from residues that have been exploited by the mycorrhizal roots and mycelium (Cairney and Burke, 1998; Haselwandter et al., 1985; Leake and Read, 1990; Read and Perez-Moreno, 2003). This source of N may explain the discrepancy between the calculated residual and the amount of N accumulated in biomass annually.

Given the discrepancy between the residual N and the amount of N added to cranberry biomass each year, it is possible that net N mineralization also supplied a portion of plant available N during the growing season. We consistently found net N mineralization was occurring, but these rates were five to ten times lower than those measured in other peat bogs in North America and Europe (Bridgham et al., 1998; Verhoeven et al., 1990). This could be attributed to the recalcitrant nature of the substrate in cranberry beds, which had a C:N ratio of around 80. The C:N ratio for peat bog studies varied between 26 to 50 (Verhoeven et al., 1990; Bridgham et al., 1998). Mineralization

commonly occurs at C:N ratios of <20 (Lutz and Chandler, 1947; Parnas, 1975), but in some wetlands it has occurred with C:N values between 60 and 100 (Malmer and Holm, 1984).

Budget indicated the importance of organic N pools as nutrient sources

Vitousek and Reiners (1975) posited that the difference between net inputs and outputs of a nutrient should be proportional to the rate at which that nutrient is incorporated into the net biomass increment for the system. If we considered the total above and belowground biomass as the increment of growth, the residual value in the study should match the net change in N held in cranberry biomass each year. However the calculated residual (29.6 kg N ha^{-1}) did not account for the change in N that accumulated in the plant biomass annually, which we estimated at ~64 kg N ha^{-1}. This difference could come from other N sources, such as fallen litter, DON in soil solution, or mineralization. The amount of N in these pools and fluxes was similar to the difference between the residual and the net increase in N held in biomass (~52 kg N ha^{-1}).

Mass balance approaches are often used to assess N inputs relative to outputs and the positive residual N could indicate that we did not fully account for all N outputs from the system in the form of leaching, effluent, denitrification, or emission. We did not measure these, but based on the similarity of cranberry systems to the study systems, we believe that literature estimates we used were reasonable.

Conclusions

We showed that there are several characteristics of current cranberry management and production that are inherently sustainable. Cranberry beds appear to be sinks for N

and C and the potential for C and N storage in soil organic material was high. In addition, our budget calculations demonstrated that large pools of organic N exist within the system that may be mobilized by ERM fungi. These ERM fungi appear to be important to the N cycle in this agroecosystem despite considerable N fertilizer inputs annually. Our budget indicated that leaching could also be a sizable N loss from cranberry beds, and future management plans may focus on N sources that have longer mean residence times and exploit the ERM capability to increase N uptake. However, more research is needed to determine how significant organic N pools are relative to plant reserves and fertilizer additions. Greenhouse and field studies that manipulate N sources, investigate N dynamics such as DON fluxes, as well as mycorrhizal status would provide valuable information to inform future production practices.

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Table 2.1 List of terms used in the nitrogen and water balance equations

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	N Source	Budget Term Abbreviation
Inputs	Soil moisture	SM
	Precipitation	P
	Irrigation	I
	Floodwater (in)	FI
	Capillary rise	C
	Mineralization	M
	Fertilizer	F
Outputs	Evapotranspiration	ET
	Floodwater (out)	FO
	Leaching	L
	Surface runoff	S
	Denitrification	D
	Emission	E
	Harvest berry and litter	H
Residual	Nutrients held in the plant and the soil	R

Table 2.2 Mean (±1 S.E.) plant nitrogen and carbon pools in biomass and litterfall for five cranberry beds.

	2004	2005	2006
	n=9	n=25	n=15
Pool	kg N ha^{-1}	kg N ha^{-1}	kg N ha^{-1}
Total vegetative biomass	165.4 ± 15.2	220.03 ± 16.0	292 ± 16.4
Aboveground vegetative biomass	44 ± 5.3	56.2 ± 2.7	59.5 ± 4.1
Belowground vegetative biomass	121.5 ± 11	163.9 ± 15.5	232.5 ± 15.5
Harvest berry	17.5 ± 1.3	19.3 ± 1.5	16.5 ± 1.4
Pool	kg C ha^{-1}	kg C ha^{-1}	kg C ha^{-1}
Total vegetative biomass	11,041 ± 823.82	10,100.39 ± 631.65	14,131.26 ± 890.07
Aboveground vegetative biomass	3691.3 ± 424.6	4451.5 ± 235.4	4755.48 ± 310.3
Belowground Vegetative biomass	7349.8 ± 548.1	5648.88 ± 535.9	9375.8 ± 829.1
Harvest berry	1642.8 ± 121.2	1812.9 ± 142.1	1543.8 ± 130.7
Flux		kg N ha^{-1}	kg N ha^{-1}
Leaf litterfall		4.0 ± 1.1	6.3 ± 0.4
Stem litterfall		22.6 ± 1.7	25.5 ± 2.5
Flux		kg C ha^{-1}	kg C ha^{-1}
Leaf litterfall		339.4 ± 86.6	511.47 ± 33.3
Stem litterfall		1810.09 ± 131.6	2053.5 ± 196.7

Table 2.3 Mean (±1 S.E.) concentrations, water volume, and N fluxes from water sources for three cranberry beds in 2006. n.a. means that data were not available. TN values were calculated by multiplying N concentration (mg L^{-1}) by volume of water and dividing by bed area. Mean bed area was 13854.33 m^2.

Source	n	N concentration	Volume	TN	DON
		mg L^{-1}	1 x 10^4 L	kg N ha^{-1}	(%)
Irrigation	8	0.9±0.21	95.6±59	7.1±2.41	59
Floodwater	5	0.7±0.13	398±52	2.1±0.39	100
Precipitation	9	1.96±0.11	693±156	9.8±0.53	n.a.
Leaching/ Capillary rise	15	2.38±1.06	730±46	12.2±5.4	14

Table 2.4 Mean (±1 S.E.) concentrations, water volume, and N fluxes for individual cranberry beds for 2006. TN values were calculated by multiplying N concentration (mg L^{-1}) by volume of water and dividing by bed area. Area of Bed 1=16856, Bed 2=9030, Bed 3=15677 m^2.Values from this table were averaged for Table 2.3.

Source	Bed	Number of samples	N Concentration	Volume	TN
			mg L^{-1}	1 x 10^4 L	kg N ha^{-1}
Irrigation	1	3	0.54±0.09	759	2.42±0.38
	4	3	0.69±0.04	542	4.15±0.25
	5	3	1.47±0.5	1568	14.75±5.01
Floodwater	1	1	0.53±0	506	1.58±0
	4	2	0.92±0.26	271	2.76±0.78
	5	2	0.57±0.13	470	1.70±0.4
Precipitation	1	5	1.96±0.2	843	9.82±1
	4	5	1.96±0.2	452	9.82±1
	5	5	1.96±0.2	784	9.82±1
Leaching	1	2	0.69±0.01	860	3.51±0.07
	4	2	7.17±0.75	461	36.59±3.84
	5	4	0.83±0.19	800	4.26±0.97

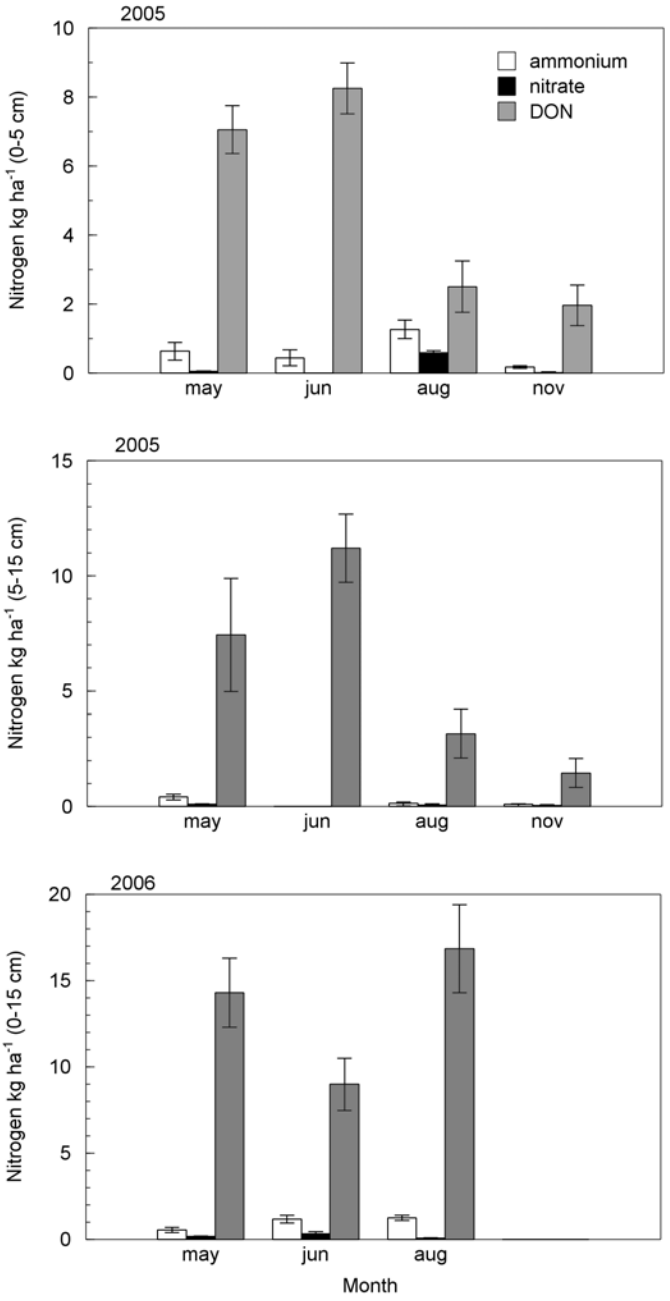

Figure 2.1 Mean extractable soil N kg ha^{-1} averaged over the growing season for sampling times in 2005 and 2006. a) The top portion of the core (0 to 5 cm) from 2005, b) The bottom of the core (5 to 15 cm) from 2005, c) The whole core 0 to 15 2006.

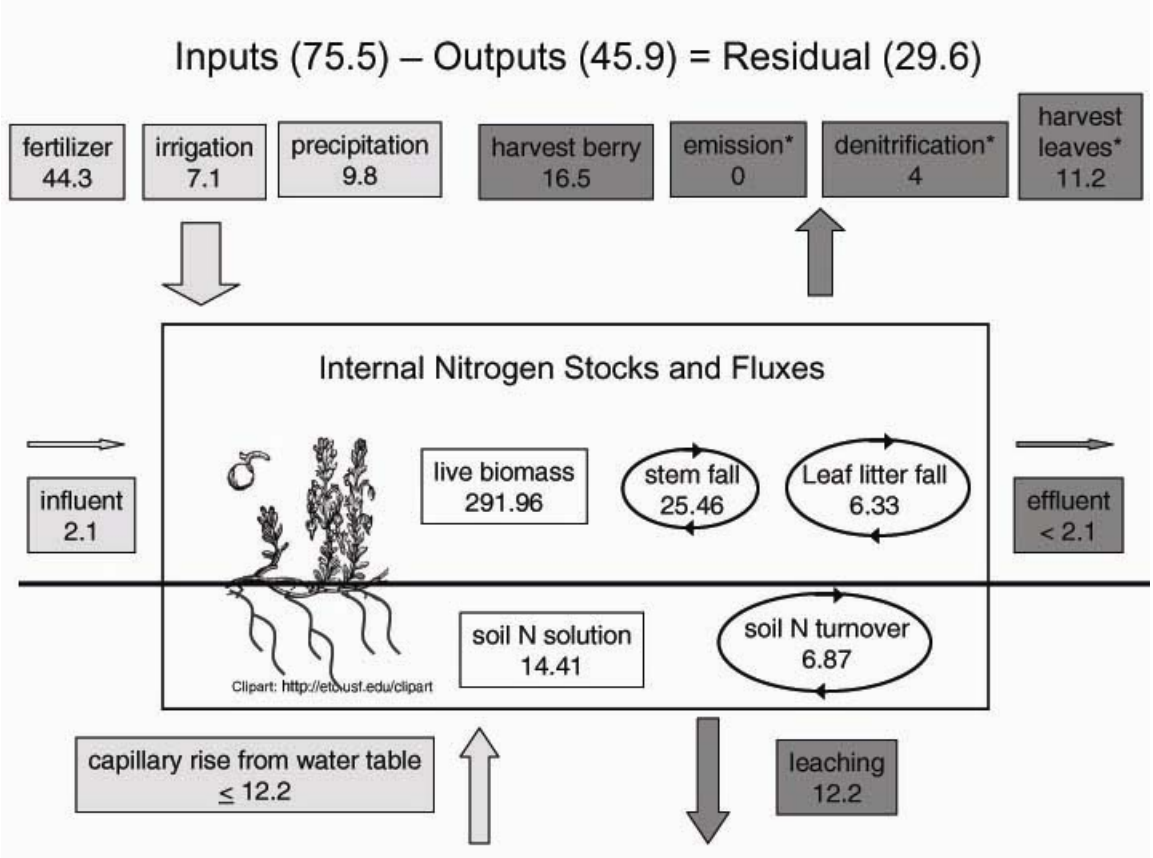

Figure 2.2 Schematic representation of annual N flows into, out of, and within the rooting zone of cultivated cranberry beds. All units are in kg N ha^{-1}, except for litterfall, stemfall, and soil turnover, which are in kg N ha yr^{-1}. Arrow thickness corresponds to flow size. Black boxes are outputs, grey boxes are inputs, white boxes are pools, and circles are fluxes.

Chapter 3. Relationships between soil nitrogen levels, ericoid mycorrhizal fungal colonization, plant nutrition and yield in the cranberry agroecosystem

Abstract

The important influence that ericoid mycorrhizal fungi (ERM) have on nitrogen cycling in the cranberry agroecosystem has been documented, but there is little information about the effect of environmental variables such as soil nitrogen (N) levels on ERM colonization rates in plantings managed for fruit production. This study was designed to investigate the site and seasonal variation in colonization rates in cultivated cranberry beds and to examine the influence of soil N levels on the variability of ERM colonization rates. We also wanted to determine if colonization rates were correlated with production variables such as fruit yield and leaf N content. Ericoid mycorrhizal colonization and extractable soil N pools were measured in cultivated cranberry beds at multiple times during the growing season in Central Wisconsin. Average ERM colonization rates (measured as percent root length colonized) ranged from 11 to 80% with significant differences in colonization rates among sites and sampling times. Soil N levels were comparable among sites, but there were seasonal differences in extractable soil ammonium, nitrate, and dissolved organic N (DON) levels. Colonization was correlated with concentrations of all soil N species'. Ammonium and nitrate concentrations were negatively correlated with ERM colonization, while DON soil pools exhibited a positive relationship. ERM colonization rates and fruit yield or leaf N content. More studies are needed to further understand the factors associated with ERM influence on nutrient cycling, including measurements of fungal biomass and fungal species

composition. Deeper understanding these issues could have important implications for management, as ERM fungi increase uptake and absorption of essential nutrients, which reduces N losses to groundwater, atmosphere, and downstream aquatic ecosystems.

Keywords: ericoid mycorrhizal fungi, *Vaccinium macrocarpon*, nitrogen fertilizer, soil nitrogen pools

Introduction

Studies in Oregon and Wisconsin have found that ericoid mycorrhizal fungi (ERM) are ubiquitous in cultivated cranberry beds (Kosola and Workmaster, 2007; Scagel, 2005a) and these fungi play a significant role in the N nutrition of cultivated cranberries by mobilizing soil nitrogen (N) for plant uptake (Stackpoole et al., 2008). Although the importance of these fungi in regulating N cycling has been noted, the environmental variables that influence colonization rates in the field are still largely unknown. Understanding factors that influence colonization rates could have important implications for improving environmental and economic sustainability of cranberry production. Since ERM fungi enable plants to increase inorganic N uptake and access organic N pools, this may allow farmers to apply less fertilizer and thereby reduce N losses to groundwater, the atmosphere, and downstream aquatic ecosystems.

Ericoid mycorrhizal colonization occurs naturally in wildland systems with low inorganic N status such as heathlands (Read and Perez-Moreno, 2003), arctic tundra (Michelsen et al., 1996) and boreal forest (Lindahl et al., 2007). Although inorganic N

levels are low in these environments, organic N levels are high. Because of their association with ERM fungi, ericaceous species living in these ecosystems can mobilize N from organic N sources and transfer N to the host plant, conferring an advantage over non-mycorrhizal species (Bending and Read, 1996; Cairney and Burke, 1998; Read and Perez-Moreno, 2003). Furthermore, when plants are provided with only organic N sources, it has been found that ERM colonization contributed to improved plant N nutrient levels (Read and Perez-Moreno, 2003). Since dissolved organic N is a large soil N pool in the cranberry agroecosystem, we investigated the relationship between these pools and ERM colonization rates in our cultivated cranberry beds.

Ammonium is considered the dominant form of inorganic nitrogen used by cranberries, and growers apply 40 to 60 kg N ha^{-1} y^{-1}, with an additional 5 to 10 kg N ha^{-1} y^{-1} available from mineralization, precipitation, and irrigation water. In a laboratory study, high ammonium levels (25 to 56 ppm) suppressed ERM colonization in cranberry (Vaccinium macrocarpon) (Stribley and Read, 1976). In addition to a perceived lack of use of nitrate by the cranberry plant, average soil nitrate levels are usually thought to be lower in cranberry beds because the acidic soil (pH ~ 5.5) limits nitrification rates (Paul and Clark, 1989). However, nitrification can persist in low pH soil, especially under conditions of high organic nitrogen availability (Booth et al., 2005). Groundwater contaminated with nitrate was also present on some cranberry farms. Kosola et al. (2007) demonstrated that ERM colonization can increase nitrate uptake rates by cranberry in hydroponic solution, but there has been no explicit study of the influence of nitrate levels on overall colonization rates in the field.

In addition to investigating the environmental conditions that may promote ERM colonization, we also were interested in the influence of ERM colonization on crop production variables such as fruit yield and leaf N content. Mycorrhizal cranberry roots can have a higher concentration of N than non-mycorrhizal plants (Scagel, 2005a), which could result in higher photosynthesis rates and increases in yield. Mycorrhizal plants of the closely related highbush blueberry (Vaccinium corymbosum L.) produced more biomass and had larger canopies relative to non-inoculated blueberries (Yang et al., 2002). Higher levels of ERM colonization have also been associated with increased fruit yield in blueberries (Powell and Bates, 1981).

The objective of this study was to test the theory that soil nutrient status influences ERM colonization rates, so we compared soil N solution levels and ericoid mycorrhizal colonization, with the expectation that higher inorganic soil N levels would correspond to less ERM colonization. We expected DON levels to have a positive relationship with ERM colonization rates and related colonization rates to leaf N content through the growing season and end-of-season yield measurements. We expected that higher colonization rates would be related to higher leaf N content and higher yields.

Materials and Methods

Cranberry bed characteristics

Data were collected from conventionally cultivated cranberry beds in Wood (44°30' N/ 90°0' W) and Juneau County (43°47' N/90°04' W) Wisconsin, USA. All beds contained the variety ‘Stevens’ and were relatively young plantings (9 to 12 years old).

We selected two beds with peat substrata and three beds with sand substrata to represent both types.

Ericoid mycorrhizal colonization

To measure ericoid mycorrhizal fungi colonization in 2004 and 2005, sub-samples of roots were taken from the top layer (1 to 2 cm) of each soil core and stored in 50% ethanol. Samples were taken in 2004 and 2005 at 4 to 5 times through the growing season from five cranberry beds. Samples were cleared and stained using the methods described in Kosola and Workmaster (2007). Root length colonization (percent root length containing ERM structures), was determined with a dissecting microscope (40x to 90x magnification), with a line intercept method (Giovannetti and Mosse, 1980).

Soil N Pools

A grid of 35 points, 5 columns by 7 rows, was laid out in four cranberry beds, which were rectangular and between 1 to 2 hectares. Columns were typically 61 m apart, and rows were 6 m apart. All samples were collected from randomly selected points on this grid. Soil solution N pools (NH_4^+, NO_3^-, and total dissolved N [TDN]) were measured from one core (5 cm deep × 8 cm diameter) taken from five randomly chosen points on the grid at four times (May, June, August, and November) in 2005. The cores were transported on ice and stored at 4ºC until analyzed (within 48 hours of collection).

A 1:10 w/v soil to 1M KCl solution was used to extract N from soil cores. Samples were shaken for 1 min, and extracted for 24 hrs at room temperature. Samples were then filtered with Whatman A/E 0.2 µm glass fiber filters. The extracts were frozen (-20°C) until analysis (Robertson et al., 1999). Ammonium measurements were

determined colorimetrically on a Labsystems Multiskan EX microplate reader (Thermo Fischer Scientific, Walthman, MA) at 650 nm absorbance using the Berthelot assay (Rhine et al., 1998). TDN measurements were made using this assay, following a persulfate digestion and a reduction with De Varda's alloy (Sims et al., 1995). We substituted phenol for 2-phenylphenol sodium salt tetrahydrate (PPS) which is a component of reagent #2 in the Berthelot assay. A precipitate consistently formed in the digested samples when PPS was used. The samples were clear when reagent #2 was made with fresh phenol, pH adjusted to 11. The absorbance values of undigested ammonium standards using phenol were identical to those using PPS (S. Stackpoole, unpublished data). Nitrate samples were reduced to ammonium with DeVarda's alloy and then analyzed. Dissolved organic nitrogen (DON) was determined by subtracting ammonium and nitrate from the TDN values. Yield estimates were collected from grower records.

Percent colonization and soil data were tested for normality and homogeneity. The colonization and soil data were analyzed Means were compared with SAS ANOVA, using transformed data when appropriate. (SAS 9.1 Statistical Software, Cary, NC). Correlations were performed using a Pearson Correlation Coeffecient in SAS. Results were considered significant with an $\alpha=0.1$ significance level. Linear regression was performed with CoStat (CoStat Statistical Software, 6.4, Monterey, CA).

Results

The range of ERM colonization rates was 11 to 80% for individual samples. Colonization was significantly different among the sampling times ($P=0.01$).

Colonization tended to be higher in April and June, rather than later in the sampling season, during August and November sampling. Colonization rates did were not significantly different between the sampling years, 2004 and 2005 (P=0.36).

Soil ammonium and nitrate levels varied significantly throughout the growing seasons, (P<0.0001) and (P<0.0001) respectively. Inorganic N levels were highest during August. During June, inorganic N levels were undetectable at all sites. Dissolved organic nitrogen dominated soil N pools, and there was a significant seasonal difference (P<0.0001). Levels were higher early in the growing season. Soil ammonium and nitrate levels were negatively correlated with colonization rates (Table 1). The relationship between DON and percent colonization was positive and significant at the 0.10 level.

There was no significant relationship between percent colonization and yield (R^2=0.05, P=0.52). There was no significant relationship between percent colonization and leaf nitrogen values (R^2=0.01, P=0.29).

Discussion

There was significant variation in ericoid mycorrhizal root length colonization rates by sampling date. Seasonal variation in fungal biomass has been linked to variation in photosynthetic rates (Olsrud et al., 2004). Lower photosynthetic rates at the end of the growing season may partially explain the lower colonization levels in August and November compared to earlier in the growing season, as peak photosynthetic rates for cranberry occur early in June and slowly decline for the rest of the growing season (Hagidimitriou and Roper, 1994). In contrast to our results, Scagel (2005) reported higher colonization rates in Coastal Oregon cranberry beds in the fall than earlier in the growing

season, but the difference in colonization rates between the early and later part of the growing season was smaller for younger bogs (2 to12 years). Our study sites fell within that age range. Colonization rates for that study could also be affected by increased root growth, which may occur later in the growing in the more temperate coastal climate of Oregon.

Our study indicated that soil N levels could play a significant role in ericoid mycorrhizal colonization rates. Higher ammonium and nitrate were negatively correlated with ERM colonization rates. A field study found that higher levels of inorganic N (~ 90 kg N ha) reduced ERM colonization rates on a related species, highbush blueberry (*Vaccinium corymbosum* L.) (Goulart et al., 1993). High levels of soluble N fertilizer also resulted in a decline of ERM colonization of rhododendron species (*Rhododendron spp.*) (Moore-Parkhurst and Englander, 1982). Our preliminary laboratory study also showed the high N levels decreased cranberry ERM colonization (Kosola et al., 2007). Considering that many ericaceous species developed symbioses with ERM in conditions of low ammonium and nitrate availability, it is not surprising that higher N levels were related to lower colonization rates. Plants do not invest heavily in hyphal root colonization, if these inorganic N sources are readily available.

In contrast, we found that higher DON pools were related positively to colonization rates. This large pool of DON could be in the form of labile amino acids, or it could be comprised of more complex tannin-bound proteins (Kalbitz et al., 2000). It is widely accepted that plants can take up amino acids directly from the soil solution (Kielland, 1994; Nasholm et al., 1998; Raab et al., 1999), but ERM's ability to mobilize N from complex organic compounds such as tannin-bound proteins, enables these organic

N pools to be nutrient sources for the plants. It would benefit the plant to invest in the ERM symbiosis to access these forms.

Although we found significant relationships between soil N status and ERM colonization rates that did not translate into higher leaf N content or higher fruit yield among our sites. Mycorrhizal colonization has been shown to increase N capture (Hodge, 2003), especially from organic material, which could result in higher plant N content (Scagel, 2005a). However, other factors, including photosynthetic rates, influence the N content of leaf material, which could override the influence of colonization on leaf N content. Mycorrhizal colonization is also just one of a multitude of factors that impacts yield as well, including shade, weed competition, fungal and insect pathogens. These factors could have confounded the positive influence that higher colonization rates could have on increasing fruit yield. Additionally, yield and N availability would most likely be strongly positively corrected when N is a limiting nutrient. Given that the cranberry growers participating in this study monitored leaf N and were regulating fertilization to maintain plant N status, it is not surprising that we didn't find a strong relationship between N and yield.

Although these results show promise in furthering our understanding of the controls on ERM colonization rates, more work needs to be done. In addition to root length colonization, it would be interesting to measure ericoid fungal biomass. Ericoid mycorrhizae belong primarily to the Ascomycotina and Basidiomycotina (Allen et al., 2003), and can form mycelia, which can have large ranges and act like conducting tissue for nutrients for the plants (Allen et al., 1995). The area covered by fungal biomass could impact nutrient uptake rates in the field.

In addition, the species of fungal symbiont and level of infection may determine the level of benefit on the plant (Goulart et al., 1993). All of our cranberry plantings were the variety Stevens, but we do not know the identities of the ERM fungi that form the symbiosis. Unlike most ectomycorrhizae, where one fungal species dominates, cells adjacent to one another can have different ericoid mycorrhizal species (Allen et al., 2003). It is possible that fungus-host specificity and inoculum may play some role in ERM colonization rates (Scagel, 2005b), and many ericoid mycorrhizal fungi are unknown and unidentified (Allen et al., 1995). Recent findings have shown that plant N is influenced by ERM fungi, and that there can be differences in uptake rates even between fungal strains that are genetically closely related (Grelet et al., 2008).

Conclusions

To our knowledge this is the first field-based study to explore the correlation of soil N status on ERM colonization that includes both inorganic and dissolved organic forms of N. Our results suggest that increased levels of inorganic N may decrease ERM colonization, while higher levels of soil organic N are related to higher colonization rates. More studies are needed to test the relationship between nutrient levels, colonization rates, and fungal biomass. Additionally, understanding host-fungus specificity, may have important implications for management, as these fungi may confer benefits such as increased nutrient access, higher growth, and better yields.

Acknowledgements

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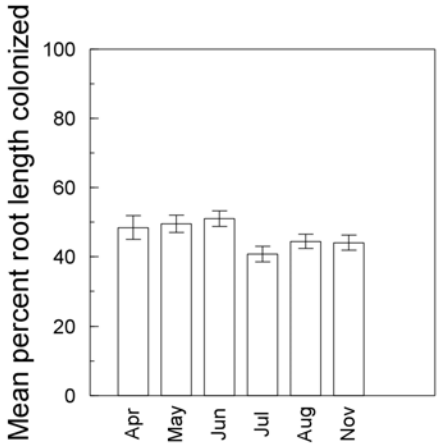

Figure 3.1 Mean percent root length colonization. Samples were taken from five cultivated cranberry beds at throughout the growing season in 2004 and 2005.

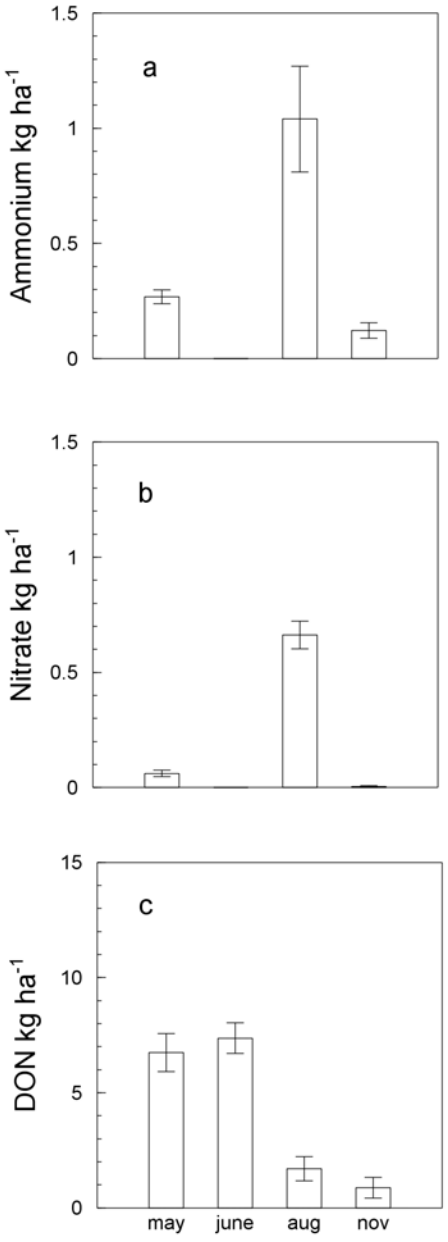

Figure 3.2 Mean extractable soil N values. (a) Extractable soil ammonium, (b) extractable soil nitrate, and (c) extractable dissolved organic nitrogen. Samples were taken from four different cranberry beds at four times throughout the growing season in 2005. Note the difference in scale between the graphs.

Table 3.1 Correlations for soil characteristics related to percent colonization in sand and peat-based beds. Soil depth (0 to 5 cm). Year 2005.

Variable	N	Pearson Correlation Coeffecient	Probability
Total Nitrogen (kg ha^{-1})	70	0.14	0.24
Ammonium (kg ha^{-1})	70	-0.22	**0.07**
Nitrate (kg ha^{-1})	70	-0.23	**0.05**
Dissolved Organic Nitrogen (kg ha^{-1})	70	0.2	**0.1**

Conclusions from Dissertation

This research project was a descriptive study that focused on exploring two key aspects of nitrogen cycling. First, we explored the potential benefits that ERM fungi may provide to production by establishing a relationship between ERM fungi and N cycling. Secondly, although inorganic N fertilizer receives the most attention by growers, we found that there were large reserves of N contained in organic pools. Because of the symbiotic relationship with ERM fungi mobilize N, these may be significant sources of N for cranberry plants. Finally, we also investigated some environmental factors that could influence ERM colonization levels in the field.

More specifically, in Chapter 1 we found that even with N additions through fertilizer, cranberries exhibited traits of plants growing in nutrient limited conditions, including litter with high C:N ratios, and slow decomposition. Additionally, using ^{15}N natural abundance measurements, we were able to provide evidence that ERM fungi mobilize N from the rooting zone. Until now, the role of ERM fungi in tightly regulating N cycling was not recognized, and most cranberry growers did not account for ERM influences on N uptake.

In Chapter 2, we demonstrated that there are several characteristics of current cranberry management and production that are inherently sustainable. First, the cranberry beds appear to be sinks for N and potential for C and N storage in soil organic material was high. Additionally, the outcome of our budget demonstrated that there were large pools of organic N recycled within the system that may be mobilized by ERM fungi.

Between 35 to 50 kg N ha^{-1} could be derived from organic N sources such as leaf litter and DON pools. Despite the potential to store nutrients, our budget indicated that leaching is also a sizable N loss from cranberry beds, and future management plans may focus on N sources that have longer mean residence times and exploit the ERM capability to increase N uptake. Finally in Chapter 3, we found that that increased levels of inorganic N between 30 to 60 kg N ha^{-1} may increase colonization, but that soil N pools had no significant relationship on ERM colonization. We also found that there was no significant difference between soil N pools in peat compared to sand substrata

These findings were derived from a descriptive study that provides incentive to continue with more research on the topics of ERM colonization and N-cycling in the cranberry agroecosystem. For example, future work could quantify the N flux through the ERM pathway to determine if and how much organic N is mobilized by ERM fungi from organic matter relative to fertilizer inputs. Future research could also investigate how significant organic N pools are relative to plant reserves and fertilizer additions. Both greenhouse and field studies that manipulate N sources, investigate N dynamics such as DON fluxes, as well as mycorrhizal status would provide valuable information to inform future production practices. Finally, more studies are needed to test the relationship between nutrient levels and colonization rates, along with fungal biomass. Understanding the influences on ERM colonization, including host-fungus specificity, may have important implications for management, as these fungi may confer benefits such as increased nutrient access, higher growth, and better yields.