MICROBIAL NITROGEN IMMOBILIZATION AS A TOOL TO MANAGE
WEEDS IN AGROECOSYSTEMS

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MICROBIAL NITROGEN IMMOBILIZATION AS A TOOL TO MANAGE
WEEDS IN AGROECOSYSTEMS

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The activity of soil microorganisms can be harnessed to promote agricultural sustainability. One such microbial approach, referred to as reverse fertilization, incorporates large quantities of high carbon:nitrogen (C:N) plant residues into the soil. The carbon amendment stimulates the growth of soil microorganisms and triggers microbial scavenging of soil nitrogen, resulting in nitrogen immobilization within microbial cells. Immobilized nitrogen is temporarily unavailable for plant uptake. Most research on this technique has involved controlling invasive plants to reestablish native plant communities that are sensitive to soils with high nitrogen fertility. The approach should be applicable to the management of agricultural weeds, particularly for nitrophilous species. We conducted one greenhouse and two field-based experiments exploring the potential of reverse fertilization as an agricultural weed management tool. We observed evidence of nitrogen immobilization in soils amended with high C:N amendments, including higher microbial biomass and respiration, and decreased plant-available soil nitrate. We also observed functional changes in the soil microbial community, primarily based on soil amendment treatment. Fungal and bacterial beta diversity were strongly influenced by the soil amendment treatment, with a consistent decrease in fungal alpha diversity in carbon amended soils. We also observed a
consistent reduction in growth and competitive ability of nitrogen-responsive weeds in carbon amended soils compared to unamended soils. We found altered functional traits of the weed community based on soil amendment treatments in our field experiment. The results from the three experiments indicate the ability of nitrogen immobilization to become a useful component of an integrated weed management program, with increasing potential as digital agriculture integrates multiple and simultaneous ecological crop management options.
BIOGRAPHICAL SKETCH

Maria Gannett grew up in Cape Cod, Massachusetts, where she first was introduced to agricultural research at the UMass Cranberry Research Station. Her first project explored the efficacy of Smolder, a formulation of *Alterneria destruens*, as a bioherbicide for dodder weed (*Cruscuta spp.*) infesting cranberry bogs. She attended the University of Mary Washington in Fredericksburg, Virginia for her undergraduate degree in environmental science. After graduating she volunteered for the Peace Corps as an agroforestry extension agent in Senegal, where she had the opportunity to work with tropical fruit crops such as mango, cashew, and citrus. When she returned, she joined Dr. Marvin Pritts’ lab and studied the effects of tillage intensity and soil amendments on soil biological health in matted row strawberry systems for her master’s degree. After graduating she continued working in the Horticulture Department as a lab manager in Dr. Jenny Kao-Kniffin’s belowground ecology lab. As a professional development activity, she took Dr. Toni DiTommaso’s weed biology and management course in fall 2017 and rediscovered her passion for weed management. She combined her interests in soil microbial ecology and weed ecology for her doctoral research exploring the potential to use microbially-mediated nitrogen immobilization as a weed management tool in agroecosystems. She was also fortunate to be selected as a graduate student fellow in the Foundation for Food and Agriculture Research fellowship program during her PhD. In this program she had the opportunity to further develop her professional soft skills with a cohort of food systems researchers from around North America. She is excited to apply her scientific knowledge and professional skills to help improve the lives of farmers and decrease the negative impacts of farming practices on ecosystems processes in her future endeavors.
Dedicated to my friends and family.

Your love is what makes all work possible.
ACKNOWLEDGMENTS

There so many people who have helped and guided me along my path that has arrived at this PhD. I have always been supported by dedicated advisors, starting with Dr. Frank Caruso, then Dr. Marvin Pritts, Dr. Jenny Kao-Kniffin, and Dr. Toni DiTommaso, and committee member, Dr. Jed Sparks. I would especially like to thank Dr. Jenny Kao-Kniffin for her unfaltering belief in me as a graduate student. Also, thank you to Dr. Lynn Johnson, who witnessed my struggle through my first statistics courses here at Cornell and who has then advised me on statistical analyses ever since. Thank you to Dr. Rebecca Dunning for all her patience and work developing my professional soft skills and providing so many opportunities to explore aspects of a professional scientific career. Much of the work I am most proud of as a PhD student was facilitated by your efforts. Around me I have also always been surrounded by supportive peers. I have been at Cornell a long time and worked with many groups of students, and consistently they have been supportive, empathetic, curious, enthusiastic, and innovative. By far, the thing I am most grateful for about my time at Cornell, has been the opportunity to get to know and work with so many inspiring peers. Thank you. I also want to thank the various agencies that have supported my work financially. I am thankful for my funding through the Horticulture Department Graduate Student Fellowship and for continued funding through the Foundation for Food and Agriculture Research fellowship program. I want to especially thank Dr. Peter Porpiglia and AMVAC for their financial support and professional mentorship. Thank you to the Cornell Atkinson Center and the Towards Sustainability Foundation who believed in the value of my research.

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CHAPTER 1
MICROBIAL NITROGEN IMMOBILIZATION AS A TOOL TO MANAGE WEEDS IN AGROECOSYSTEMS

1.1 Abstract

The activity of soil microorganisms can be harnessed to promote agricultural sustainability. One such approach, referred to as reverse fertilization, incorporates large quantities of high carbon:nitrogen (C:N) amendments into the soil. The carbon stimulates the growth of soil microorganisms and triggers microbial scavenging of soil nitrogen, resulting in nitrogen immobilization within microbial cells. Immobilized nitrogen is temporarily unavailable for plant uptake. Most research on this technique has involved controlling invasive plants to reestablish native plant communities that are sensitive to soils with high nitrogen fertility. We reviewed the literature on reverse fertilization and found that perennials, legumes, and plants with higher root:shoot ratios were less responsive to the high C:N amendments compared with annuals, plants that do not fix nitrogen, and plants with lower root:shoot ratios. Based on these contrasting responses, we believe there is potential to improve and increase the use of reverse fertilization as a weed management tool in agroecosystems. This tool, along with other ecology-based methods, can be incorporated into farm-scale prescriptive management systems to improve the sustainability of crop production.

1.2 Introduction

The modification of soil carbon to nitrogen ratios (C:N) is a technique that has been used with considerable success in native prairie and forest restoration research to control weedy and invasive plant establishments. The mechanism of weed control is
based on microbial immobilization of N, whereby microbes immobilize N in the short-term within microbial biomass. If microbial C and N are not sequestered in soil as more stable forms, cell turnover and decreased microbial N use efficiency have the potential to rapidly release N back into plant available and unstable forms (Mooshammer, Wanek, Hämmerle et al., 2014; Robertson et al., 2019; Zhang et al. 2021). This strategy has the potential to be effective in agroecosystems, which are often populated with N-responsive (nitrophilous) weeds that thrive in high N soils (Moreau, 2014). In annual cropping systems, many of the most troublesome (e.g., glyphosate-resistant and abundant) weeds are nitrophilous (Costea et al., 2004; Little et al., 2021). Some of these weed species can outcompete crops for N and have higher N use efficiencies, which can lead to lower crop yields in fertilized soil relative to unfertilized soil at high weed densities (Di Tomaso, 1995; Little et al., 2021). Management practices that modify soil C:N could have major impacts on crop production of these systems (Mohler et al., 2021). If the timing of N immobilization occurs during the critical period of weed control, this temporary reduction in inorganic N availability for plant uptake could be a powerful ecological tool for agricultural weed management.

Developing ecological methods for weed control is increasingly necessary given the escalating prevalence of herbicide-resistant weed populations globally (Liebman and Gallandt, 1997). Currently, herbicides are the primary method of weed control in North America (Shaner and Beckie, 2013) and their use continues to increase (Kniss, 2017). As weed species are repeatedly exposed to herbicides, they develop resistance through selection on naturally occurring genetic variations that promote survival (Vencill et al., 2017). There are now 263 species of weeds resistant to at least one
herbicide worldwide, and many of those are resistant to multiple modes of action. A herbicide’s mode of action is the sequence of events at the cellular level that leads to plant death. Multiple herbicides can share the same mode of action, so developing resistance to one mode of action can reduce the efficacy of multiple herbicides (Gunsolus and Curran, 1999). When a weed biotype develops resistance to one mode of action, growers will often transition to herbicides with a different mode of action, which can accelerate the evolution of weed populations resistant to multiple modes of action. This feedback loop promotes an overreliance on herbicides, sometimes referred to as a “herbicide treadmill” that can be difficult to escape (Binimelis et al., 2009).

Remaining on the herbicide treadmill is a strategy that could be effective if new modes of action could be developed frequently. However, there has now been a ~30-year period in which no new herbicide modes of action have been discovered, highlighting the vulnerability of agricultural systems reliant on herbicides as the exclusive means of weed control (Gaines et al., 2021). Once a mode of action has been lost for a weed population, few new options exist. The most effective way to manage increasing populations of herbicide-resistant weeds is through an integrated weed management program, combining a variety of weed management strategies with different mechanisms of control (Gage et al., 2019; Mortensen et al., 2012). Commonly used non-chemical weed management strategies include seedbank management, tillage, crop rotations, cover cropping, timing of critical periods of weed control, and precision fertilization (Shaner and Beckie, 2013). However, adoption of integrated weed management approaches remains relatively low. More research on existing strategies and the development of novel strategies may allow growers to manage herbicide-
resistant weed populations with combinations of non-chemical approaches (Harker and O’Donovan, 2013).

In this review paper, we describe an ecological approach to manage N-responsive weed species. This approach uses high C:N amendments to trigger microbial immobilization of inorganic N. We selected this weed management tool, which has been described in the restoration ecology literature, for its potential to be impactful in agriculture. The first section of this review describes how the soil environment is altered by high C amendments, why these changes encourage native plant growth, and how competitive dynamics shift. The second section summarizes and evaluates the literature on C additions for weedy and invasive plant management in restoration ecology. The final section of this review discusses how the method can be applied to precision weed management scenarios in agricultural systems.

Several early restoration ecology studies referred to the strategy of adding C to the soil to immobilize N as “reverse fertilization” or “soil impoverishment” (Hopkins, 1998; Morgan, 1994). These terms are no longer in use, but the concept is emerging with relevance to farming systems, so we adopt the term “reverse fertilization” to name this useful tool for weed management in specific cropping systems.

1.3 Theoretical applications of soil C:N modification

1.3.1 Microbial stoichiometry and soil nutrient immobilization

The goal of reverse fertilization is to immobilize N and other nutrients by stimulating microbial growth through C addition. Soil nutrient cycling is primarily controlled by soil microbial mineralization, a process in which microbes release extracellular enzymes that deconstruct organic molecules to access the minerals that
support their growth (Mooshammer, Wanek, Zechmeister-Boltenstern et al., 2014; Schimel and Bennett, 2004). Carbon is the primary element needed to produce energy for microbial growth, maintenance, and enzyme production (Moorhead et al., 2012; Sinsabaugh et al., 2013); therefore, C is the primary driver of microbial activity. When C is available, other nutrients may limit microbial growth. The required ratio of C to another essential nutrient is known as the threshold element ratio (TER) for that nutrient (Mooshammer, Wanek, Zechmeister-Boltenstern, et al., 2014). Below the TER, microbial growth is limited by C, so other nutrients are excreted back into the soil (mineralized). Above the TER, other nutrients are limiting and are invested into microbial growth (immobilized). For N, the threshold element ratio ranges approximately between 20:1 and 25:1 (Mooshammer, Wanek, Hämmerle et al., 2014; Sinsabaugh et al., 2013). This ratio has been measured empirically, but can also be calculated by dividing the soil microbial community biomass C:N \( \left( B_{C:N} \right) \) by its C use efficiency \( (CUE) \) and multiplying by its assimilation efficiency \( (A) \) (Equation 1).

\[
TER = A \left( \frac{B_{C:N}}{CUE} \right) \tag{1}
\]

The global average C:N of the soil microbial community is approximately 7:1 (Cleveland and Liptzin, 2007; Mooshammer, Wanek, Zechmeister-Boltenstern et al., 2014), the average C use efficiency is approximately 0.3 (Mooshammer, Wanek, Zechmeister-Boltenstern et al., 2014; Sinsabaugh et al., 2013), and the microbial assimilation efficiency is usually estimated at 1 (Mooshammer, Wanek, Hämmerle et al., 2014; Sinsabaugh et al., 2013). Therefore, the threshold element ratio is approximately 23:1. Most agricultural soils have lower C:N, around 20:1 or lower, which promote N mineralization. However, N is immobilized when amendments with
high C:N, such as sawdust or straw, are added to the soil in quantities sufficient to raise the bulk C:N above 23:1. This N immobilization effectively lowers the plant-available N until enough C has been utilized to bring the bulk C:N back below 23:1 (Vinten et al., 2002).

1.3.2 Potential benefits of immobilizing plant-available N

In natural systems, reducing plant-available N often helps native species compete with invasive species that are responsive to high soil N levels. Available N has been increasing globally with increasing fossil fuel combustion and agricultural intensification (Galloway, 2008). Local disturbances, such as vegetation removal, can further increase local N availability (Norton et al., 2007). Plant-available N is an important selective factor in plant succession. Most early-seral plant species have traits associated with high soil N availability and later-seral plant species have traits associated with lower soil N availability (Vitousek and White, 1991). Invasive plants are often early-seral, weedy species. Therefore, the high N levels observed following a disturbance may benefit weedy species and displace the previously dominant native plant community (Davis et al., 2000). Without environmental restoration, the native plant community may struggle to reestablish even when propagules are available (Stylinski and Allen, 2001) and disturbance can lead to long-term non-native species invasions (Baker, 1974; Pyšek and Richardson, 2007; Rejmanek and Richardson, 1996). Invasions reduce plant diversity (Pyšek et al., 2012) and cause large-scale ecosystem changes (Mack et al., 2000; Vitousek et al., 1996). Thus, the restoration of environmental conditions promoting native plant species growth is critical to native plant reestablishment (Norton et al., 2007) and ecosystem health. Immobilizing N with
C amendments may be one way to restore favorable conditions for native plant species by modifying competitive interactions between native and invasive species.

1.3.3 Nitrogen immobilization changes competitive dynamics

Nitrogen immobilization may influence plant competition through several mechanisms. First, NH$_4^+$ and small organic N compounds become the predominant sources of plant-available N above the TER as NO$_3^-$ becomes less available (Mooshammer, Wanek, Hämmerle et al., 2014; Schimel and Bennett, 2004). Although plants can use multiple N sources, species differ in their preferences (Magalhaes and Huber, 1991) and degrees of plasticity (Tylova-Munzarova et al., 2005). Consequently, species may respond differently when NH$_4^+$ and organic N replace NO$_3^-$ as the predominant sources of plant-available N. The second mechanism involves differential effects of total plant-available N on plants with different functional traits. In high-resource soils, aboveground competition for light is the key to plant survival (Booth et al., 2003; Wilson and Tilman, 1993), so successful plants exhibit traits such as high dispersal ability, high rates of leaf and shoot production, and high leaf photosynthetic capacity (Redente et al., 1992; Tilman, 1985). In resource-poor soils, plant survival is more strongly related to a plant’s ability to utilize scarce abiotic resources (Booth et al., 2003; Wilson and Tilman, 1993) and depends on belowground investment and root production (Redente et al., 1992; Tilman, 1985). Additionally, immobilized N is eventually released back into the soil, but this release is relatively slow because N residence times are longer in microbial necromass relative to plant
Figure 1.1. Illustration of plant competitive dynamics under reverse fertilization. Images in the left column represent plant growth in unamended soils and images in the right column represent plant growth in high carbon:nitrogen-amended soils. Time since treatment increases down each column (T2 = second timepoint, T3 = third timepoint). Within each box, the plant on the left represents a slow-growing perennial grass (e.g., *Elymus canadensis*) and the plant on the right represents a nitrogen-responsive (nitrophilous), fast-growing, annual forb (e.g., weedy *Amaranthus* spp.). Dissolved inorganic nitrogen is indicated by N.
material. (Wang et al., 2020). Therefore, decreasing the amount of available soil N changes the relative fitness of plants according to their growth strategies (Chapin, 1980). Therefore, reverse fertilization may favor plants with higher root:shoot or plants that accumulate N slowly, relative to plants with a high initial demand for N.

To summarize the theory underlying reverse fertilization, adding amendments with high C:N to the soil can increase bulk C:N above the threshold element ratio. Soil microbial growth is stimulated by the added C, resulting in N immobilization. The decrease in plant-available N alters competitive dynamics, potentially favoring plants that more readily acquire N from sources other than NO$_3^-$ or invest more resources in belowground growth and slow resource capture (Fig. 1.1).

### 1.4 From theory to practice

#### 1.4.1 Reverse fertilization in restoration ecology

To evaluate whether reverse fertilization can cause biologically meaningful changes, we reviewed the literature on reverse fertilization used in restoration ecology. To help visualize species response patterns from this literature, we have tabulated the information. Basic study design information is summarized in Table 1.1. When possible, individual plant species responses to C amendments are included in Tables 1.2 and 1.3, depending on how the species responded to increased C:N soils. Plant species that showed improved growth in C-amended soil or reduced growth in N-amended soil were included in Table 1.2, whereas plant species that showed reduced growth in C-amended soil or improved growth in N-amended soil were included in Table 1.3. Many studies do not report plant species growth responses individually, so we summarized major
results from all the reviewed studies in Appendix Table 1.1. Below we discuss results presented in these tables and additional results from the reviewed literature.

Table 1.1. Restoration ecology papers featuring soil carbon amendment as a tool to restore native plant populations in disturbed habitats.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Carbon source</th>
<th>Carbon amount (kg C ha$^{-1}$ yr$^{-1}$)</th>
<th>Years (of application, data collection)</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>McLendon and Redente, 1992</td>
<td>sucrose</td>
<td>1,600</td>
<td>3, 3</td>
<td>Sagebrush in Colorado</td>
</tr>
<tr>
<td>Morgan, 1994</td>
<td>sawdust and sucrose</td>
<td>8,400 kg sugar-C + 80 L sawdust-C</td>
<td>1, 1</td>
<td>Tallgrass prairie in Manitoba, Canada</td>
</tr>
<tr>
<td>Young et al., 1998</td>
<td>sucrose</td>
<td>470</td>
<td>6, 6</td>
<td>Northwestern Great Basin in California</td>
</tr>
<tr>
<td>Zink and Allen, 1998</td>
<td>bark and straw</td>
<td>3 cm thick</td>
<td>1, 3</td>
<td>Coastal sagebrush in Southern California</td>
</tr>
<tr>
<td>Reever Morghan and Seastedt, 1999</td>
<td>sawdust and sucrose</td>
<td>1,500</td>
<td>3, 3</td>
<td>Prairie in Colorado</td>
</tr>
<tr>
<td>Paschke et al., 2000</td>
<td>sucrose</td>
<td>1,600</td>
<td>4, 4</td>
<td>Shortgrass steppe in Colorado</td>
</tr>
<tr>
<td>Alpert and Maron, 2000</td>
<td>sawdust</td>
<td>5,850</td>
<td>1, 2</td>
<td>Coastal grassland in Northern California</td>
</tr>
<tr>
<td>Torok et al., 2000</td>
<td>sawdust and sucrose</td>
<td>700</td>
<td>1, 1</td>
<td>Sand dunes in the Danube-Tisza Region, Hungary</td>
</tr>
<tr>
<td>Cione et al., 2002</td>
<td>bark and leaf mixture</td>
<td>2.5 cm thick</td>
<td>1, 3</td>
<td>Coastal sage scrub in Southern California</td>
</tr>
<tr>
<td>Blumenthal et al., 2003</td>
<td>sawdust and sucrose</td>
<td>840 – 33,460</td>
<td>1, 2</td>
<td>Prairie in Minnesota</td>
</tr>
<tr>
<td>Corbin and D'Antonio, 2004</td>
<td>sawdust</td>
<td>2,340</td>
<td>2, 2</td>
<td>Coastal grassland in California</td>
</tr>
<tr>
<td>Perry et al., 2004</td>
<td>cover crop and sawdust</td>
<td>6.1% C</td>
<td>1, 0.48</td>
<td>Minnesota wetland sedge meadow, greenhouse</td>
</tr>
<tr>
<td>Baer et al., 2004</td>
<td>sawdust</td>
<td>21,450</td>
<td>1, 3</td>
<td>Prairie in Kansas</td>
</tr>
<tr>
<td>Huddleston and Young, 2005</td>
<td>sawdust</td>
<td>1,560</td>
<td></td>
<td>Agate desert in Oregon</td>
</tr>
<tr>
<td>Prober et al., 2005</td>
<td>sucrose</td>
<td>8,400</td>
<td>2, 2</td>
<td>Grassy white box woodlands in New South Wales, Australia</td>
</tr>
</tbody>
</table>
1.4.2 Soil C amendments can immobilize N

Previous studies suggest that C amendments can immobilize N, but only when large amounts of C are added to the soil. Effective C amendment rates were approximately 8,000 to 16,000 kg C ha\(^{-1}\) yr\(^{-1}\) (Table 1.1) (Alpert and Maron, 2000; Blumenthal et al., 2003; McLendon and Redente, 1992; Paschke et al., 2000; Prober et al., 2005) and high inherent soil N levels made achieving sufficient N immobilization more difficult (Busby et al., 2019; Morgan, 1994). In most studies that measured soil N,
reduced N availability in C-amended soil was observed (Blumenthal et al., 2003; Baer et al., 2004; Cole et al., 2021; Eschen et al., 2007; Knauf et al., 2021; McLendon and Redente, 1992; Paschke et al., 2000; Perry et al., 2004; Reever Morghan and Seastedt, 1999; Uddin et al., 2020) with a few exceptions (Busby et al., 2019; Yelenik et al., 2016). Nitrogen immobilization decreased over time as the added C source was used by the soil microbial community (Blumenthal et al., 2003). If N was not immobilized long enough for native plant species to reestablish successfully, weedy species could reestablish in amended areas (Reever Morghan and Seastedt, 1999). Overall, increasing the bulk soil C:N above the threshold element ratio appeared to be difficult, but possible with large quantities of added C.

1.4.3 Variability in invasive plant management after soil C amendments

Carbon amendments reduced the growth of invasive species more frequently than they reduced the growth of native species (Table 1.3). However, this pattern was not universal, with many studies showing that C amendments did not exclusively improve native species growth and hamper invasive species growth (Blumenthal et al., 2003). The most common outcome is that C amendments reduced both native and weedy invasive species growth but had larger negative effects on weedy species (Alpert and Maron, 2000; Baer et al., 2004; Busby et al., 2019; Corbin and D’Antonio, 2004; Eschen et al., 2007; McLendon and Redente, 1992; Morris and Gibson-Roy, 2018; Paschke et al., 2000; Yelenik et al., 2016). In some rare cases, native and invasive plant growth experienced similar declines (Reever Morghan and Seastedt, 1999). In parallel studies that increased soil N and C, invasive plant growth, that have an evolutionary history of low nutrient availability, increased under increased N, but there was little effect of any
amendment (N or C) on native plant growth or the continental grassland species (Cole et al., 2021; Knauf et al., 2021).

Several longer-term studies found that plant communities grown in C-amended soil transitioned to a later seral stage one to four years faster than plant communities grown in unamended soil (Eschen et al., 2007; McLendon and Redente, 1992; Paschke et al., 2000). Nitrogen amendments had the opposite effect, enabling early-seral species to maintain their dominance in the plant community over time (Paschke et al., 2000). Species richness often increased in C-amended soil (Baer et al., 2004; McLendon and Redente, 1992) and both species richness and diversity declined in N-amended soil (Baer et al., 2004). In one study, native species richness increased when low amounts of C were added to the soil but decreased at high rates of soil C amendment (Morris and Gibson-Roy, 2018).

1.4.4 Soil C amendments alter plant competition dynamics

We examined the restoration ecology literature to evaluate the hypothesis that N-fixing species, perennial species, and species with high root:shoot would outcompete other species in C-amended soils. These characteristics increase belowground investment, which would improve a plant’s ability to utilize scarce abiotic resources (Blumenthal et al., 2003).

Few reverse fertilization studies have reported data on individual N-fixing species, but these studies consistently show increased growth of N-fixing species in C-amended soils (Table 1.2). Not all these N-fixing species were native plants. For example, the native Hawai’ian tree, *Acacia koa* A.Gray (koa), and the invasive tree, *Morella faya* (Aiton) Wilbr (firetree), both had reduced survival in N-amended plots.
(Yelenik et al., 2016). Another study that did not report species-specific responses also found that legumes grew better in C-amended soils (Eschen et al., 2007).

Most species-specific data on responses to C amendments were collected for perennial species, especially perennial grasses (Tables 1.2 and 1.3). Several studies reported improved growth of perennials in C-amended soil (McLendon and Redente, 1992; Paschke et al., 2000; Prober, 2005), regardless of whether the plant was invasive or native (Blumenthal et al., 2003). No invasive perennial grasses showed improved growth in C-amended soils (Table 1.2), but both native and invasive perennial grass species showed reduced growth in C-amended soils (Table 1.3). Corbin and D’Antonio (2004) reported that C amendments helped a native perennial grass compete with exotic annuals during the first year of treatment. However, in subsequent years, native perennial grass growth was reduced due to competition with both perennial and annual weeds, regardless of C amendment treatment (Corbin and D’Antonio, 2004). Root:shoot of individual plant species were generally not reported, but several studies found that plants with higher root:shoot were favored in C-amended soils (Paschke et al., 2000; Perry et al., 2004). Conversely, plants with lower root:shoot were favored in N-amended soils (Paschke et al., 2000).
Table 1.2. Plant species that showed improved growth in carbon-amended soil or reduced growth in nitrogen-amended soil, relative to other treatments. Species that showed improved growth in one study but no response in another study were still classified as improved growth. Species are categorized by growth habit, life history, and native/invasive status. Species listed are from papers in restoration ecology where soil carbon amendments were used as a tool to restore native plant populations in disturbed habitats (Table 1.1).

<table>
<thead>
<tr>
<th>Native plant species</th>
<th>Annual</th>
<th>Annual/biennial/perennial</th>
<th>Perennial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graminoid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forb or Vine</td>
<td></td>
<td>Trifolium dubium&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>Trifolium pratense&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Subshrub</td>
<td></td>
<td></td>
<td>Machaeranthera asteroids, Monarda bradburiana</td>
</tr>
<tr>
<td>Shrub/Tree</td>
<td></td>
<td></td>
<td>Ericameria ×bolanderi</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Invasive plant species</th>
<th>Annual</th>
<th>Annual/biennial/perennial&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Perennial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forb or Vine</td>
<td></td>
<td>Hibiscus trionum</td>
<td>Cirsium arvense, Convolvulus arvensis,</td>
</tr>
<tr>
<td>Shrub/Tree</td>
<td></td>
<td>Silene latifolia</td>
<td>Morella faya&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Combination of annual, biennial, and perennial life histories
<sup>2</sup>Nitrogen fixer
Table 1.3. Plant species that showed **reduced** growth in carbon-amended soil or improved growth in nitrogen-amended soil, relative to other treatments. Species are categorized by growth habit, life history, and native/invasive status. Species listed are from papers in restoration ecology where soil carbon amendments were used as a tool to restore native plant populations in disturbed habitats (Table 1.1).

<table>
<thead>
<tr>
<th>Native plant species</th>
<th>Annual</th>
<th>Annual/biennial/perennial</th>
<th>Perennial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Graminoid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forb or Vine</td>
<td></td>
<td>Helianthus annuus</td>
<td>Centella asiatica</td>
</tr>
<tr>
<td>Shrub/Tree</td>
<td></td>
<td></td>
<td>Chenopodium oahuense</td>
</tr>
<tr>
<td><strong>Forb or Vine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive plant species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Forb or Vine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrub/Tree</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Combination of annual, biennial, and perennial life histories*
1.5 Potential for reverse fertilization as an ecological tool in agriculture

The final section of this review considers how reverse fertilization could be incorporated into regenerative agriculture. This strategy requires theoretical and applied ecology to assume a central role in decision-making about agroecosystem management. In addition, it requires site-specific datasets to be merged into multi-scale ecological models that can support agroecological management tools. Although there are several challenges associated with using models to make management recommendations, modeling has great potential to simplify complex decision-making processes and may identify more sustainable options (Addison et al., 2013; Schuwirth et al., 2019). In prescriptive agricultural systems, farm-management recommendations and decisions are driven by the analysis of farm-specific data (Smith, 2020). These decisions can involve conventional agricultural practices such as fertilizer and pesticide applications, but could also involve ecological practices such as reverse fertilization. In the latter case, farm-specific data could be used to determine the optimal timing and location for C inputs. Prescriptive agricultural technology is currently used by agricultural companies for tasks such as monitoring crop health; packing fruit; identifying management needs related to nutrition, weed pressure, and disease incidence; and prescribing variable-rate pesticide and fertilizer applications (Jung et al., 2021). There is great potential to incorporate ecology-based practices into algorithms used for prescriptive agriculture and thereby generate more sustainable management recommendations (Jung et al., 2021).

Incorporating ecology-based practices into prescriptive agriculture will be more difficult than current uses of prescriptive agriculture. Agroecological datasets are large
and complex datasets, often referred to as ‘big data’, defined by having high volume (size of the data), velocity (flow of the data), variety (lack of standardized structure of the data), and veracity (accuracy of the data) (Coble et al., 2018). Running these data through complex models requires high computing power; infrastructure which is often currently lacking in many rural agricultural communities. However, technologies to incorporate ecology-based management practices such as reverse fertilization are in development. There are growing calls to bring full connectivity to rural communities with fiberoptic cables or satellite connections (Crist, 2022). It is predicted that increased internet connectivity could greatly increase farmer output and productivity (Goedde et al., 2020; LoPiccalo, 2021) and could also help conserve fossil fuels, water, and soil health. Technologies to quickly and affordably collect detailed farm-scale data needed to make recommendations about where reverse fertilization would be useful on a field-scale are being developed. For example, visible-near infrared diffuse reflectance spectroscopy technology now has the capacity to measure soil organic matter, mineral composition, clay content, and soil moisture with one reading (Stenberg et al., 2010). Weedy patches in fields can be identified with spectral sensors attached to unmanned aerial vehicles (Esposito et al., 2021). Specific plant species (Lottes et al., 2017; Sanders et al., 2019) and even herbicide-resistant biotypes of certain species can now be identified using these technologies (Reddy et al., 2014; Shirzadifar et al., 2020). These data will need to be merged. For example, plant traits such as N-responsiveness of each identified weed species and crop nutrition needs can be overlaid onto a field’s soil map. Figure 1.2 visualizes how this process could help identify locations likely to benefit from reverse fertilization. These locations may include areas where the weed
community is more nitrophilous than the crop, areas where herbicide-resistant weeds dominate, or areas with weed seedbanks comprised of difficult-to-control weed species.

For reverse fertilization to be adopted at large scales, general ecological models will need to be refined and integrated with site-specific information. Substrate decomposition models, which are continually improving, can be combined with site-specific soil data to ensure that adequate C is applied to immobilize N throughout the critical period of weed control (Kyker-Snowman et al., 2020; Robertson et al., 2019; Zhang et al., 2021). As sequencing costs continue to decrease, it may become increasingly feasible to gather data on the composition of soil microbial communities (Wetterstrand, 2021). These compositional data can help improve site-specific model predictions of substrate decomposition rates, especially as we better understand how microbial diversity and life histories affect soil traits such as C use efficiency (Domeignoz-Horta et al., 2020; Malik et al., 2020). Improved understanding of vertical differences in soil composition through the soil profile – both mineral and biological – will help determine the most effective way and form of C add to the soil (Hsiao et al., 2018). Carbon amendments can then be injected or incorporated through localized tilling into soils using GIS-guided technologies. Such improvements in modeling, on-farm data collection, data analysis, and actuation are likely to facilitate the adoption of reverse fertilization and other ecology-based tools in the near future.
Figure 1.2. Vision for prescriptive agriculture that incorporates ecological theory into field management practices. I.) Farm-scale data layers include plant species variables with functional traits, including nitrogen responsiveness, in addition to crop, soil, and environmental data. II.) Field data are integrated into ecosystem models to generate predictive scenarios for weed management. III.) Precision application technology helps guide carbon amendment inputs to locations where reverse fertilization is predicted to be effective.
REFERENCES


Booth, B.D., Murphy, S.D., Swanton, C.J., 2003. Interactions between populations I: Competition and allelopathy. In B.D. Booth, S.D. Murphy, C.J. Swanton (Eds.) Weed ecology in natural and agricultural systems (pp.121). CABI.


https://doi.org/10.1614/WT-D-12-00109.1


https://scholarsarchive.byu.edu/wnan/vol65/iss4/9


https://www.jstor.org/stable/3658632

https://www.jstor.org/stable/3505888


https://doi.org/10.1111/j.1365-2486.2011.02636.x

https://doi.org/10.1002/ps.3755

semiarid seral species to nitrogen and phosphorus gradient. Plant and Soil. 140,
127–135. https://doi.org/10.1007/BF00012814

https://doi.org/10.1046/j.1526-100X.1999.07106.x

Rejmanek, M., Richardson, D.M., 1996. What attributes make some plant species

Robertson, A.D., Paustian, K., Ogle, S., Wallenstein, M., Lugato, E., Cotrufo, M.F.,
2019. Unifying soil organic matter formation and persistence frameworks: The

Sanders, J.T., Everman, W., Austin, R., Roberson, G., Richardson, R., 2019. Weed
species differentiation using spectral reflectance land image classification.
Proceedings of SPIE, Advanced Environmental, Chemical, and Biological
Sensing Technologies 11007. https://doi.org/10.1117/12.2519306

paradigm. Ecol. 85, 591–602. https://doi.org/10.1890/03-8002

Schuwirth, N., Borgwardt, F., Domisch, S., Friedrichs, M., Kattwinkel, M., Kneis, D.,


https://doi.org/10.2307/1478913

Tylova-Munzarova, E., Lorenzen B., Brix H., Votruba, O., 2005. The effects of
NH$_4^+$ and NO$_3^-$ on growth, resource allocation and nitrogen uptake kinetics of
https://doi.org/10.1016/j.aquabot.2005.01.006

invasibility of nutrient enriched plant community invaded by *Phragmites

Vencill, W.K., Nicholas, R.L., Webster, T.M., Soteres, J.K., Mallory-Smith, C.,
Toward and understanding of resistance development and the impact of
herbicide-resistant crops. Weed Sci. 60, 2–30. https://doi.org/10.1614/WS-D-
11-00206.1

N immobilization/mineralization kinetics for cellulose-, glucose-, and straw-
amended sandy soils. Biol. and Fertil. of Soils. 36, 190–199.
https://doi.org/10.1007/s00374-002-0524-y

invasions as global environmental change. Am. Sci. 84, 468–478.

H.H. Shugart, D.B. Botkin (Eds.), Forest Succession Concepts and


https://doi.org/10.1046/j.1526-100x.1998.00617.x
CHAPTER 2
ALTERING MICROBIAL COMMUNITY STRUCTURE AND FUNCTION BY MANIPULATING SOIL RESOURCE AVAILABILITY TO MANAGE AGRICULTURAL WEEDS

2.1 Abstract

We examined the use of carbon amendments to stimulate microbial immobilization of soil nitrogen for weed control. We buried 80 19-L pots in a research farm field and added sawdust and sucrose to soils as a high carbon treatment and used unamended soils as a control. We examined eight different weed species separately, and measured plant growth, soil carbon, available nitrate, microbial carbon and nitrogen, and microbial community composition after 11 weeks of treatment. The carbon amendments altered plant-microbial competition, resulting in reduced biomass for most weed species. The carbon amended soils had higher microbial biomass carbon and nitrogen, slower nitrogen cycling, and less available soil nitrogen, indicating enhanced nitrogen immobilization. The carbon treatment altered the beta diversity of soil fungi and bacteria and reduced fungal alpha diversity estimated by the Shannon index. The study results indicate that high carbon substrates can be used to modify plant-microbial competition for soil nitrogen with important implications for developing sustainable weed management practices.

2.2 Introduction

Managing weeds in agricultural fields is key to food security globally. It is estimated that without managing weeds, 50% of maize yield (Soltani et al., 2016) and 52% of soybean yield (Soltani et al., 2017) would be lost in the United States (U.S.) and
Canada. Herbicides are the primary tool used to manage weeds in the U.S. (Shaner and Beckie, 2013), and their use continues to increase (Kniss, 2017). However, as weed species are repeatedly exposed to herbicides, they develop resistance through selection on naturally occurring genetic variation that promote survival (Vencill et al., 2012). As the efficacy of one herbicide declines, growers can shift to another, but if use and selection pressure remain high, weeds will continue to develop resistance to these new herbicides quickly (Gaines et al., 2021). Developing management tools that modify the soil environment of weeds can be an effective alternative method.

The soil microbial community is a critical component of the biophysical environment in which crops are grown. Soil carbon additions stimulate the growth of the soil microbial community and microbial biomass because carbon is often limiting in bulk soil environments (Zak et al., 1994; Kallenbach and Grandy, 2011). As the primary driver of microbial activity, carbon affects microbial mineralization and immobilization processes impacting plant nutrition (Schimel and Bennett, 2004). The ratio of carbon to another nutrient in the soil that determines mineralization/immobilization is referred to as the threshold element ratio (TER) for that nutrient (Mooshammer et al., 2014b). For nitrogen, the TER is typically between 20:1 to 25:1 (Sinsabaugh et al., 2013; Mooshammer et al., 2014a) and when carbon levels are below the TER, microbial growth is limited by carbon, so nitrogen is excreted back into the soil (mineralized). When carbon levels are above the TER, nitrogen is limiting and invested into microbial growth (immobilized). Most agricultural soils promote nitrogen mineralization because their carbon:nitrogen ratio (C:N) is 20:1 or below. One potential way to reduce weed
growth is to decrease plant available nitrogen by raising the soil C:N above the TER and stimulating microbial nitrogen immobilization.

The microbially-mediated nitrogen immobilization strategy has been previously described as “reverse fertilization” in a study involving the restoration of native plant species in disturbed natural habitats (Hopkins, 1998). In natural systems, reducing plant-available nitrogen can promote native plant species growth while suppressing growth rates of invasive plants in high-resource environments (Grime, 1977). Reverse fertilization has the potential to be applied in agricultural settings because many agricultural weed species are highly responsive to nitrogen (i.e., nitrophilous) (Blackshaw et al., 2003; Moreau et al., 2014; Little et al., 2021).

Historically, microbial responses to changing resource availability have been difficult to study (Fierer et al., 2007; Nannipieri et al., 2020). However, microbial communities vary in their ecological functioning due to differences in the generation of microbial products (Schimel and Schaeffer, 2012), bacterial:fungal ratios (Strickland and Rousk, 2010; Malik et al., 2016), and in carbon use efficiency rates (Sinsabaugh et al., 2013). Therefore, to understand microbially-mediated processes such as immobilization/mineralization and long-term carbon storage, it is critically important to understand drivers of taxonomic and functional changes to microbial community composition (Manzoni and Porporato, 2009; Schimel and Schaeffer, 2012; Zhu et al., 2020). To contextualize microbial community assembly, several established ecological concepts have been applied to microbial ecology (Prosser et al., 2007). Microbial communities may assemble based on life history strategies such as r- and K- selection (oligotrophic-copiotrophic) (Fierer et al., 2007; Morrison et al., 2018) or microbial
communities may assemble in a framework similar to Grime’s competitor–stress-tolerant–ruderal theory, where environments select for the predominance of high growth yield, stress tolerant, or competitive resource acquisition microbes (Malik et al., 2020). Microbial resource availability may also change microbial diversity, increasing diversity as resource constraints are lifted and then decreasing diversity as resource-specialists begin to dominate (Prosser et al., 2007). High soil carbon applications should stimulate the growth of different microbial taxa unequally, changing soil function.

We examined modifying plant-microbial competition for soil nitrogen through applications of high carbon soil amendments. We hypothesized that carbon resources would control the growth of the soil microbial community, resulting in greater nitrogen immobilization within actively growing microbial cells and thereby decrease plant-available nitrogen and reduce the growth of nitrogen-responsive plant species, many of which are aggressive weeds and invasive species in a broad range of high nitrogen soil ecosystems. Carbon resources would also change microbial community composition and increase taxonomic diversity. To test this prediction, we added high carbon amendments in the form of sawdust and sucrose to soil and grew eight important agricultural weed species in pots buried in a research field. We monitored microbial growth and nitrogen availability to confirm that nitrogen was immobilized. We assessed changes in microbial community composition through amplicon sequencing of the bacterial and fungal communities and measured plant growth responses to the carbon treatment.

2.3 Materials and methods
2.3.1 Experimental design

Soil was removed from 80 19-L plastic pots buried in the Caldwell Field Research Complex in Ithaca, NY, USA in June 2019 (42°27′02.3″ N, 76°27′41.1″ W). To improve drainage, pots had three holes drilled in the bottom and drainage tile was installed. Pots has originally been filled with a Mardin channery silt loam collected from Mt. Pleasant (42°27′50.2 N 76°22′24.5″ W) for a previous experiment, but all soil was removed from pots, mixed with 11 19-L buckets of sand, and homogenized by hand. Pots were refilled with a layer of 8 L of homogenized soil and then a layer of 10 L of treatment soil. Treatment soil either remained unamended or was amended with 14.3 g (9 mL) of sucrose, 286 g (2.6 L) of dry weight sawdust, and soil to a total volume of 10 L. Sawdust was 48% C and sucrose was 42% C, for a total of 144 g of C amendment. PVC rings 20 mm high and 50 mm wide were placed in the pots. Mesh bags (L’eggs everyday suntan; Hanesbrands, Rural Hall, NC, USA) containing resin beads (Dowex Marathon MR-3 Mixed Ion Exchange Resin; Serv-A-Pure, Bay City, MI, USA) were placed inside the rings at a depth of 20 cm, directly below the treatment soil.

A pre-counted number of seeds were sown in pots on July 9, 2021. *Abutilon theophrasti* seeds were scarified by dipping in boiling water for 5 seconds before planting and all other seeds were directly seeded. All seed had been collected from local agricultural fields and germination rates were all above 28.2% (n=5) when grown on moist Whatman 1 filter paper in a petri dish at 22°C for 7 days (Table 2.1), except *Ambrosia artemisiifolia*, which had a lower germination rate (7%) over a longer period (1 month).
Table 2.1. Weed seeds used in a field pot study tracking species growth and emergence in unamended soils or soils with high carbon amendments. The number of seeds and the method used to count the seeds planted in each pot are recorded below, as well as percent germination in the lab.

<table>
<thead>
<tr>
<th>Weed</th>
<th>Counting Method</th>
<th>Seeds (number)</th>
<th>Speed</th>
<th>Sensitivity</th>
<th>Germination test (mean %, standard deviation)</th>
<th>Maximize plants per pot (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abutilon theophrasti</td>
<td>Seed counter</td>
<td>50</td>
<td>70</td>
<td>27</td>
<td>80.4, 6.6</td>
<td>5</td>
</tr>
<tr>
<td>Amaranthus powellii</td>
<td>Hand</td>
<td>100</td>
<td>n/a</td>
<td>n/a</td>
<td>36.8, 2.9</td>
<td>2</td>
</tr>
<tr>
<td>Amaranthus rudis</td>
<td>Hand</td>
<td>100</td>
<td>n/a</td>
<td>n/a</td>
<td>67.8, 3.8</td>
<td>2</td>
</tr>
<tr>
<td>Ambrosia artemisiifolia</td>
<td>Hand</td>
<td>50</td>
<td>n/a</td>
<td>n/a</td>
<td>7, 4.8</td>
<td>1</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>Seed counter</td>
<td>100</td>
<td>68</td>
<td>23</td>
<td>28.2, 3.9</td>
<td>1</td>
</tr>
<tr>
<td>Echinochloa crus-galli</td>
<td>Seed counter</td>
<td>50</td>
<td>68</td>
<td>18</td>
<td>93.6, 4.6</td>
<td>7</td>
</tr>
<tr>
<td>Ipomoea hederacea</td>
<td>Seed counter</td>
<td>50</td>
<td>70</td>
<td>30</td>
<td>81.6, 5.0</td>
<td>7</td>
</tr>
<tr>
<td>Trifolium repens</td>
<td>Seed counter</td>
<td>100</td>
<td>55</td>
<td>18</td>
<td>71.6, 7.1</td>
<td>5</td>
</tr>
</tbody>
</table>

Seedling emergence was recorded starting on July 16 and then once weekly until August 26, when all weeds were thinned to no more than a specified number of plants per pot as noted in Table 2.1. The number of weeds was maintained at the specified level until harvest. Only target species were allowed to grow in the pots. All other plants were removed by hand. The coefficient of velocity of germination was calculated according to the equation in Al-Ansari and Ksiki (2016) (Equation 1).

\[
CVG = \frac{N_1 + N_2 + \cdots + N_i}{100 \times N_1T_1 + N_2T_2 + \cdots + N_iT_i} \times 100
\]  

(1)

\[N_i = \text{number of seeds germinated every day}\]

\[T_i = \text{number of days from seeding corresponding to } N\]

2.3.2 Sample collection
Soils were collected as intact columns to the base of the pot on September 25 with a 20 mm wide soil push probe (Classic soil probe; Oakfield apparatus company, Oakfield, WI, USA). Three cores were collected and aggregated from each pot. One core was collected and then discarded before collecting samples from that pot to “rinse” the probe. Samples were stored in a cooler in the field and then transferred to a cooler set to 3°C (Setpoint temperature control model SP-322; Goldline, N. Kingstown, RI, USA). Each soil sample was later sieved through a 2-mm pore size sieve and aliquoted into two bags. One bag was returned to the cooler for microbial biomass extractions and measurements of soil moisture, pH, and C:N. The other bag was frozen for DNA amplification.

Plant samples were collected on September 26 by first cutting aboveground biomass at the base of the plant, then carefully extracting the roots after using a shovel to loosen the soil. Resin bags were also removed and soil brushed off the bag. Roots and resin bags were stored individually in the cooler until processed. Aboveground biomass and roots were rinsed to remove soil before drying in an oven at 60°C until dry. All aboveground plant material was run through a Wiley Mill (Digital variable speed ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ, USA) with a 2-mm mesh sieve.

Plant and soil C and N were measured using soil that had been ground into a fine powder. Soil was ground using a mortar and pestle and plant material was ground using a ball mill (Mixer mill MM 400; Retsch, Haan, Germany). About 30 mg and 3 mg of powdered soil and plant material, respectively, were weighed into tin capsules and submitted to the Cornell Stable Isotope Lab for total C, N, $^{13}$C, and $^{15}$N analysis on an
isotope ratio mass spectrometer (Delta V, Thermo Scientific, Waltham, MA) interfaced to an elemental analyzer (NC2500, Thermo Scientific, Waltham, MA).

To measure nitrate availability, resin bags were placed in acid-washed sample cups and 50 mL of 2 M KCl was added to each. Cups were shaken for 2 h at 180 rpm on a reciprocal shaker (E6000 Medium-Duty; Eberbach Corporation, Ann Arbor, MI, USA) then filtered through Whatman 1 filter paper. Filtrate was collected and stored frozen at -20°C until analysis. To analyze filtrate, 10 μL of sample was mixed with 160 μL of vanadium cocktail solution and incubated for 2 hours at 37°C. Reflectance was recorded at 540 nm on a microplate reader (Synergy HT; BioTek, Winooski, VT, USA). Samples were diluted 1 part sample to 20 parts 2 M KCL to ensure that sample levels were within detection limits.

To measure microbial biomass, two 8-g aliquots of fresh soil were weighed into tin capsules and sample cups. The sample cup aliquots were shaken with 50 mL of 0.05 M K₂SO₄ at 180 rpm for 2 h before filtering through Whatman 1 filter paper. Filtrate was collected and stored frozen at -20°C until analysis. The tin capsule aliquots were arranged in a desiccator so that all capsules were exposed to the air inside the desiccator, with a damp paper towel to maintain humidity, and a 50-mL beaker in the center of the tins. The beaker was filled with 15 mL of chloroform and the desiccator was sealed. The chloroform was brought to a boil with a vacuum pump three separate times to ensure fumigation of samples inside the desiccator. Fumigation was stopped after 20 hours. Samples were then transferred to sample cups with 50 mL of 0.05 M K₂SO₄ and shaken and extracted using the method described for the unfumigated aliquot.
Soil moisture was calculated by weighing a 12-g subsample of fresh soil into a tin cup and drying at 105°C in an oven (Isotemp Oven; Fisher Scientific, Suwanee, GA, USA) for 24 h before reweighing.

To measure pH, 3.0 ± 0.1 g of air-dried soil was mixed with 6 mL of deionized water in a 15 mL falcon tube and shaken on a reciprocal shaker at 180 rpm for 60 minutes. Tubes were removed from the shaker and allowed to sit for at least 10 minutes before pH measurements were taken with a pH meter (Combo meter; bluelab, Tauranga, New Zealand).

2.3.3 Microbial DNA extraction and amplification

DNA was extracted from approximately 0.2 g of soil using the Qiagen DNeasy soil DNA extraction kit (Beverly, MA, USA) following manufacturer instructions, without 5-minute incubation steps. Bacterial and fungal community composition were assessed using high-throughput amplicon sequencing of the V3-V4 region of the 16S rRNA gene (16S) and the ITS2 region of the fungal internal transcribed spacer gene (ITS) region, respectively. To amplify DNA, Polymerase Chain Reactions (PCR) were run on a Bio-Rad C1000 Thermal Cycler (Hercules, CA, USA), using 1 µL of 1:2 and 1:10 dilutions of the extracted DNA for the 16S and ITS regions, respectively. Reactions included 8 µL of 5 PRIME HotMasterMix (5 PRIME Inc, Gaitherburg, MD); 1 µL or 0.5 µL of both forward and reverse primers for the 16S or ITS regions, respectively; 1 µL of DMSO for ITS regions; and sterile water for a total volume of 20 µL. Primers were at a concentration of 10 µM. To target the 16S region, 341F and 805R primers were used (Herlemann et al., 2011). These primers included overhangs for index attachment. PCR cycling conditions for amplification were: 94°C
for 2 minutes, then 25 cycles of 94°C for 20 seconds, 55°C for 20 seconds, and 72°C for 30 seconds, followed by a final elongation at 72°C for 3 minutes. To target the ITS region, the ITS1F and 58A2R primers were used (Gardes and Bruns, 1993; Martin and Rygiewicz, 2005). PCR cycling conditions for amplification were: 94°C for 3 minutes, then 40 cycles of 94°C for 20 seconds, 45°C for 30 seconds, and 72°C for 45 seconds, followed by a final elongation at 72°C for 5 minutes.

The full volume of initial amplicons was cleaned using MagBio HighPrep PCR beads (MagBio Genomics, Gaithersburg, MD, USA) following manufacturer instructions. Unique two-barcode index primers were attached to the cleaned amplicons in reactions including 5 µL of sample, 2.5 µL of water, 12.2 µL of Q5 High Fidelity 2X Master Mix (New England Biolabs Inc., Ipswich, MA, USA) and 2.5 µL of forward and reverse primers containing the barcodes and the complementary side of the overhanging region of the amplicons. PCR cycling conditions for indexing were: 98°C for 1 minute, then 8 cycles of 98°C for 15 seconds, 55°C for 30 seconds, and 72°C for 20 seconds, followed by a final elongation at 72°C for 3 minutes. The amount of DNA in each barcoded amplicon was then normalized using the SequalPrep Normalization Kit (Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer instructions. Amplicons were pooled separately for 16S and ITS amplicons, combining 6 µL of each normalized sample. Pools were concentrated using a Centri-vap DNA concentrator (Labconco, Kansas City, MO, USA) and then run on a 1.2% agarose gel. As a final cleaning step, bands of expected size were excised from the gel and processed using the Wizard SVG gel and PCR clean-up system (Promega, Madison, WI, USA) following manufacturer instructions, running the sample through the filter twice. Cleaned,
indexed, and pooled samples were sequenced on the Illumina MiSeq at the Cornell Genomics Facility (Ithaca, NY, USA) using a 500-cycle MiSeq Reagent Kit v.2 for the ITS pool and a 600-cycle MiSeq Reagent Kit v.3 for the 16S pool.

2.3.4 DNA sequence processing

Raw sequence reads were imported into Qiime2 (Bolyen et al., 2019) where all sequence preparation occurred. Reads were demultiplexed, and paired ends were merged and trimmed of primers using q2-demux. Then sequences were denoised into amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). ASVs were clustered using de-novo q2-vsearch using 97% shared identity as the cutoff, to create a table of assigned operational taxonomic units (OTUs). OTUs were then assigned taxonomy based on a Naive Bayes Classifier trained on the UNITE_ver8_dynamic database for ITS and on the 99% greengenes database 13_8 for 16s (DeSantis et al., 2006; Abarenkov et al., 2010). Finally, OTU abundance and taxonomy tables were exported along with representative sequences for subsequent analysis in R version 4.1.2 (R core team, 2021).

2.3.5 Statistical analyses

Plant characteristics were analyzed using a linear model with weed species, soil amendment, and their interaction as the independent variables and plant growth metrics (total, per plant, and aboveground biomass, or coefficient of velocity of germination), as the dependent variables (function lm, in package stats, version 4.1.2). If necessary, dependent variable data were square root transformed to satisfy assumptions of homogeneity of variance, normality of residuals, and normal variance of predicted residuals, but true means and standard errors are graphed to convey biological
significance. Since different weed species are assumed to have different biomasses, post-hoc analyses of plant growth metrics were performed for each weed species using estimated marginal means comparisons with a Bonferroni correction for multiple means comparisons (function `emmeans`, in package `emmeans`, version 1.7.1-1). Soil parameters were analyzed similarly, with the same independent variables, but soil parameters (pH, soil moisture, nitrate, and C:N ratio) as the dependent variables.

To prepare data for microbial community analyses, samples with less than 1,500 bacterial reads or less than 8,000 fungal reads were removed, data were randomly subsampled to provide an equal number of sequence reads for all samples (rarefied), and feature counts were converted to percentages. Percent abundance was converted to a Bray-Curtis distance matrix (function `vegdist`, in package `vegan`, version 2.5-7) (Dixon, 2003). A principal coordinates analysis (PCoA) was performed on the distance matrix (function `cmdscale`, in package `stats`, version 4.1.2). A Permutational Multivariate Analysis of Variance (PERMANOVA) using 999 permutations was run to determine significant differences between treatments with pseudo-F-ratios (function `adonis`, in package `vegan`, version 2.5-7). Shannon diversity was calculated (function `diversity`, in package `vegan`, version 2.5-7) and then analyzed using the same linear model as described above with treatment, weed species, and their interaction as independent variables. To identify sequences predictive of treatment, the `randomForest` machine learning algorithm was used (function `randomForest`, in package `randomForest`, version 4.6-14). These identified sequences were then placed into a heatmap with columns clustered based on soil treatment (function `pheatmap`, in package `pheatmap`, version 1.0.2). The importance of these sequences was verified by
determining microbial sequences associated with treatment variables using the function Microbiome Multivariable Association with Linear Models 2.0 (Maaslin2) (function \textit{Maaslin2}, in package \textit{Maaslin2}, version 1.8.0) (Mallick et al., 2021).

\textbf{2.4 Results}

\textbf{2.4.1 Soil nitrogen immobilization impacts on plant growth}

Plant growth response, soil nitrogen availability, and microbial growth and community composition all changed in response to soil carbon amendments. There was an effect of soil amendment treatment on plant growth. Both total aboveground (df=7, $F$-value=5.0, p=0.0003) and root (df=7, $F$-value=6.0, p<0.0001) biomass per pot varied based on the interaction of the amendment treatment with the plant species (Fig. 2.1). \textit{Abutilon theophrasti}, \textit{Amaranthus powellii}, \textit{Amaranthus rudis}, \textit{Echinochloa crus-galli}, and \textit{Ipomoea hederacea} had higher biomass in unamended soil than carbon amended soil (p<0.05). When there was a change in aboveground growth, there was a corresponding change in root growth. \textit{Chenopodium album} only showed higher biomass in unamended soil at p<0.1. Amendment treatment did not affect biomass in \textit{Ambrosia artemisiifolia} or \textit{Trifolium repens}. Root:shoot varied based on plant species (df=7, $F$-value=3.2, p=0.008), although no plants had different root:shoot according to a Tukey’s post-hoc analysis of difference.
Figure 2.1. Mean weights for eight common weed species planted in pots filled with high-carbon-amended soils or unamended soils. Total aboveground and root weights were measured per pot. Within parameter and plant species, asterisks indicate a significant effect of amendment treatment on weight (**, p-value < 0.05; *, p-value < 0.1). Most, but not all, plant species had reduced growth in carbon-amended soils. An analysis of variance indicated that root:shoot varied based on plant species (p<0.05), although no plants had significantly different root:shoot according to a Tukey’s post-hoc analysis of difference.
Plant emergence was faster in unamended pots than carbon amended pots (df=1, F-value=8.9, p=0.004) (Fig. 2.2). *I. hederacea* emerged faster than any other plant species (p<0.05). *A. theophrasti* and *E. crus-galli* emerged faster than *A. rudis, C. album* and *T. repens* (p<0.05). $\delta^{15}$N of the plants was consistently higher in unamended pots than carbon amended pots (df=1, F-value=16.25, p=0.0002) (Fig. 2.3). In carbon amended pots, *A. powellii* had higher $\delta^{15}$N than *I. hederacea* and *T. repens*, and *T. repens* had lower $\delta^{15}$N than all other species except for *I. hederacea* and *A. rudis* (p<0.05). In unamended pots, *A. powellii, A. theophrasti,* and *C. album* had higher $\delta^{15}$N than *I. hederacea* (p<0.05).

Figure 2.2. Mean seedling emergence rates (CVG) for eight common weed species planted in pots filled with high-carbon-amended soils or unamended soils. Species labeled with different letters are significantly different from other species. Slower emergence rates in carbon-amended soils are indicated by the significant treatment p-value (p<0.05).
Figure 2.3. Difference between the atmospheric nitrogen-15 isotope and plant nitrogen-15 isotope ($\delta^{15}N$) of eight weed species planted in pots filled with high-carbon-amended soils or unamended soils. The $\delta^{15}N$ of the plants is acting as a phytometer to measure plant-available nitrogen. The lower $\delta^{15}N$ in plants grown in carbon-amended soils (indicated by the significant treatment p-value) is evidence of slower nitrogen cycling in those soils. Species labeled with different letters are significantly different from other species. The interaction between species and treatment is not significant.

2.4.2 Soil characteristics

There was an effect of amendment treatment on soils collected at harvest (Fig. 2.4). Soil moisture (df=1, F-value=313.7, p<0.0001), soil C:N (df=1, F-value=132.9, p<0.0001), microbial biomass carbon (df=1, F-value=18.6, p<0.0001), and microbial biomass nitrogen (df=1, F-value=133.7, p<0.0001) were all higher in carbon amended soils than unamended soils (Table 2.2). Nitrate accumulation in resin bags was higher in unamended soils than carbon amended soils (df=1, F-value=16.0, p=0.0002). The pH of the soil did not differ based on amendment treatment (p=0.75) or plant species (p=93).

Table 2.2. Mean values of soil variables and p-values from a type II ANOVA of a linear model with a carbon amendment treatment, weed species, and their interaction as independent variables, and soil metrics as dependent
variables: pH, soil moisture, nitrate from resin beads, carbon:nitrogen ratio, microbial biomass carbon, and microbial biomass nitrogen. The nitrate linear model was analyzed with a type III ANOVA since the interaction was significant. Plants were grown in pots buried in a field in Ithaca, NY.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Amendment treatment</th>
<th>Mean value in carbon amended soil</th>
<th>Mean value in unamended soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.75</td>
<td>6.38</td>
<td>6.40</td>
</tr>
<tr>
<td>Soil moisture (%)</td>
<td>&lt;0.0001</td>
<td>19.6 a</td>
<td>14.8 b</td>
</tr>
<tr>
<td>Carbon:nitrogen</td>
<td>&lt;0.0001</td>
<td>17.3 a</td>
<td>12.7 b</td>
</tr>
<tr>
<td>Microbial biomass carbon (µg·g soil⁻¹)</td>
<td>&lt;0.0001</td>
<td>376 a</td>
<td>204 b</td>
</tr>
<tr>
<td>Microbial biomass nitrogen (µg·g soil⁻¹)</td>
<td>&lt;0.0001</td>
<td>39 a</td>
<td>22 b</td>
</tr>
<tr>
<td>Nitrate (ppm NO₃·g soil⁻¹)</td>
<td>&lt;0.0001</td>
<td>0.03 b</td>
<td>0.38 a</td>
</tr>
</tbody>
</table>

Microbial biomass nitrogen also varied based on plant species (df=7, F-value=4.6, p=0.0003). Soils growing *A. theophrasti* had higher microbial biomass nitrogen than *A. artemisiifolia*, *E. crus-galli*, *I. hederacea*, and *T. repens*. There was also an interaction between plant species and soil treatment for nitrate accumulation in resin bags (df=7, F-value=5.5, p<0.0001). There was no difference in nitrate accumulation between species in carbon-amended soil. However, in unamended soil, the least amount of nitrate accumulated in *A. theophrasti* and *I. hederacea*. Both plant species had lower nitrate than *A. artemisiifolia*, *A. rudis*, *C. album*, and *T. repens*, but *A. powellii* was not lower than *I. hederacea*. *Trifolium repens* and *C. album* had the highest nitrate accumulation, with higher accumulation than *E. crus-galli* and *A. powellii* (*T. repens* only).
2.4. Principal coordinates analysis of soil traits from pots filled with high-carbon-amended or unamended soils. Different plant species growing in the pots are indicated with different colors and different shapes indicate soil type. Points that are closer to one another have more similar soil trait values. Arrows indicate the direction and influence of the soil values on point distribution. Carbon amended soils are associated with higher soil C:N, higher microbial biomass nitrogen (N) and carbon (C). Unamended soils are associated with higher nitrate values collected in resin bags throughout the growing season.

2.4.3 Soil microbial community structure and function

A total of 6,476 bacterial and 2,631 fungal sequences were observed in the 80 soil samples, with a mean of 14,939 bacterial reads and 25,706 fungal reads per pot. The alpha diversity of the bacterial community, measured by the Shannon diversity index, was higher in carbon amended soil than in unamended soil for pots growing *T. repens* (Fig. 2.5). Bacterial evenness and richness were unaffected by treatment or weed species. The alpha diversity of the fungal community measured by evenness, richness,
and Shannon diversity, was lower in the carbon-amended soil regardless of plant species. Additionally, soils growing in *I. hederacea* had higher fungal richness than soils growing in *E. crus-galli*. The Principal Coordinates Analysis (PCoA) based on a Bray-Curtis distance matrix of both bacterial (Fig. 2.6a) and fungal (Fig. 2.6b) sequences showed a clear separation between unamended and carbon amended pots. These results were confirmed by a PERMANOVA analysis that indicated a difference in bacterial (df=1, F Model=4.52, p=0.001) and fungal (df=1, F Model=29.73, p=0.001) beta diversity between the treatments. The PERMANOVA analysis also identified a difference in beta diversity for bacterial sequences based on the interaction of plant species and treatment (df=7, F Model=1.25, p=0.001).

Microbial sequences that contributed to these differences between microbial community composition and diversity were identified through two different methods. The Random Forest machine learning algorithm identified the OTUs that accurately classified the soil treatment based on a series of randomly generated OTU-based decision trees most frequently. Higher Gini Indexes indicated that the OTU is more strongly predictive of soil treatment. The out-of-bag error was 5.48% and 1.25% for bacterial and fungal predictors, respectively. Maaslin2 is an R-based software developed to assess multivariable associations of microbial community features with complex metadata using generalized linear and mixed models (Mallick *et al.*, 2021). Maaslin2 evaluates each feature for association with specified metadata, and then p-values are adjusted for multiple hypothesis testing. In this case, OTUs were evaluated for association with soil amendment treatment. The OTUs with adjusted p-values < 0.05 were significantly different based on treatments.
Figure 2.5. Mean diversity index (evenness, richness, and Shannon diversity) of bacterial and fungal communities from pots filled with high-carbon-amended or unamended soils. An * indicates that the diversity is significantly different based on soil treatment with a p-value < 0.05. Bars labeled with different letters indicate that diversity is significantly different based on weed species with a p-value < 0.05.
Figure 2.6. Principal coordinates analysis of bacterial (A) and fungal (B) communities from pots filled with carbon-amended or unamended soils. Different plant species growing in the pots are indicated with different shapes and different colors indicate soil amendment type. Points that are closer to one another have more similar microbial community structures and dependent variables that are significantly different based on a permutational multivariate analysis of variance, with pseudo p-values < 0.05, are indicated on the graph. For both bacteria and fungi, communities are more similar to one another based on soil treatments.
The top 20 most predictive bacterial OTUs (Fig. 2.7a) and 20 most predictive fungal OTUs (Fig. 2.7b, highest Gini Indexes) from Random Forest were mapped in heatmaps. The samples were divided into carbon-amended and unamended pots. Most of the 20 identified bacterial OTUs were more abundant in carbon-amended pots, with the exception of five: 986dd6, 83ba76, afb89c, 6f9970, and 9f2971. All but one of the fungal OTUs (091e08) were more abundant in carbon amended pots than unamended pots.

The OTUs identified with Maaslin2 were also displayed in heatmaps divided by soil treatment (Appendix Fig. 2.1). All but three of the identified bacterial OTUs (0c4101, 9f2971, and 986dd6) and four of the identified fungal OTUs (f80b26, 091e08, a6ea3d, and c9d7b1) were more abundant in carbon-amended soil. A comparison of the top 20 most predictive OTUs identified by Random Forest with the top 20 most significant OTUs identified by Maaslin2 revealed that 12 bacterial OTUs and 15 fungal OTUs were identified by both methods (Table 3). Of these 12 bacterial OTUs, two were unclassified, one was identified to the class of Alphaproteobacteria (Proteobacteria), three were identified to the family of Rhodobacteraceae (Alphaproteobacteria, Rhodobacterales), one was identified to the genus of Helicobacter (Campylobacterales, Helicobacteraceae) and the remaining five were identified to the genus BD2-6 (Nitrospirales, Thermodesulfovibrionaceae). One of the BD2-6, and one of the Rhodobacteraceae were more abundant in unamended soil. Of the 15 fungi, five were unclassified, two were identified to the phylum Basidiomycota, one was identified to the phylum Ascomycota, one was identified to the order of Sebacinales (Basidiomycota,
Agaricomycetes), two were identified to the genus of *Subulicystidium* (Trechisporales, Hydnodontaceae), one was identified to the genus of *Podosora* (Sordariales, Lasiosphaeriaceae), and three were identified to the species level: *Botryotrichum atrogriseum* (Sordariales, Chaetomiaceae), *Leucosporidium drummii* (Leucosporidiales, Leucosporidiaceae), and *Acanthostigma filiforme* (Tubeufiales, Tubeufiaceae). *Botryotrichum atrogriseum* was more abundant in unamended soil.
Figure 2.7. Heatmap showing relative abundance of bacterial (A) and fungal (B) operational taxonomic units (OTUs) from pots filled with carbon-amended or unamended soils. The OTUs shown are the 20 most predictive of soil amendment type based on Gini Indexes calculated from a Random Forest algorithm. The phylogenetic order and class (for bacteria and fungi, respectively) and Gini Index of each OTU is shown to the right. Most of the OTUs are more abundant in carbon-amended soil than in unamended soil for both bacteria (15 of 20) and fungi (19 of 20). The 6-digit identifiers of each OTU correspond to the same 6-digit identifier in Table 2.3.
Table 2.3. Top 20 most predictive bacterial and fungal operational taxonomic units (OTUs) identified by the Random Forest algorithm for classifying soil as amended with carbon or unamended. OTUs marked with an * indicate that these OTUs were also identified by the Microbiome Multivariable Association with Linear Models 2.0 algorithm as statistically significant based on soil treatment. The 6-digit identifiers of each OTU correspond to the same 6-digit identifier in Figure 2.7.

<table>
<thead>
<tr>
<th>Number</th>
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<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
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<tbody>
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<td>Rhodobacterales</td>
<td>Rhodobacteraceae</td>
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<td>Alphaproteobacteria</td>
<td>Nitrospirales</td>
<td>[Thermodesulfovibrionaceae]</td>
<td>BD2-6</td>
<td></td>
</tr>
<tr>
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<td>Nitrospira</td>
<td>Epsilonproteobacteria</td>
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<td>Helicobacter</td>
</tr>
<tr>
<td>c6db1c*</td>
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<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Rhodobacterales</td>
<td>Rhodobacteraceae</td>
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<tr>
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<td>Nitrospira</td>
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<td>Nitrospira</td>
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<tr>
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<td>Alphaproteobacteria</td>
<td>Rhodobacterales</td>
<td>Rhodobacteraceae</td>
<td></td>
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<td>Proteobacteria</td>
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<tr>
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<td>Agaricomycetes</td>
<td>Trechisporales</td>
<td>Hyphomicrobiaceae</td>
<td>Hyphomycetidae</td>
<td>Subulicystidium</td>
</tr>
</tbody>
</table>

59
| 133b83* | Fungi | Basidiomycota | Microbotryomycetes | Leucosporiales | Leucosporidiaceae | Leucosporidium | drummii |
| d8172d* | Fungi | Basidiomycota | Agaricomycetes | Trechisporales | Hydnodontaceae | Subulicystidium |
| 7a8ff0* | Fungi | Agaricomycetes | Trechisporales | Hydnodontaceae | Subulicystidium |
| 5a71a9* | Fungi | Agaricomycetes | Trechisporales | Hydnodontaceae | Subulicystidium |
| 8b3cc0* | Fungi | Sordariomycetes | Sordariales | Lasiosphaeraceae | Podospora |
| 9c1a6c* | Fungi | Sordariomycetes | Sordariales | Lasiosphaeraceae | Podospora |
| d83ad7* | Fungi | Sordariomycetes | Sordariales | Lasiosphaeraceae | Podospora |
| 772b78* | Fungi | Agaricomycetes | Sebacinales |
| 53aa49* | Fungi | Agaricomycetes | Sebacinales |
| 16522d* | Fungi | Agaricomycetes | Sebacinales |
| a76b65* | Fungi | Agaricomycetes | Sebacinales |
| 1806a9 | Fungi | Sordariomycetes | Sordariales | Lasiosphaeraceae | Arniun |
| 21b386* | Fungi | Sordariomycetes | Sordariales | Lasiosphaeraceae | Arniun |
| d3d473* | Fungi | Basidiomycota | Chaetomiaceae | Botryotrichum | atrogriseum |
| 347439 | Fungi | Sordariomycetes | Sordariales | Chaetomiaceae | Botryotrichum | atrogriseum |
| 89f042 | Fungi | Ascomycota | Pezizomycotina | Pezizomycotina | Pezizomycotina | Candelabrum | brocchiatum |
| 0e566c | Fungi | Ascomycota | Sordariomycetes |
| c22d5e | Fungi | Basidiomycota | |
| 091e08* | Fungi | Ascomycota | Sordariomycetes | Sordariales | Chaetomiaceae | Botryotrichum | atrogriseum |
2.5 Discussion

2.5.1 Decreased soil nitrogen availability and plant growth suppression

Reducing plant-available nitrogen negatively affected the growth of several of the weed species included in our experiment. Both *Amaranthus powellii* and *Amaranthus rudis* consistently had reduced plant growth in carbon amended soils. Many *Amaranthus* species are highly nitrophilous (Costea et al., 2004; Moreau et al., 2014) and may be especially sensitive to nitrogen immobilization. These species are among the most problematic weeds in field crops across the U.S., contributing to significant yield reductions in maize, cotton, and soybean systems (Knezevic et al., 1994; Bensch et al., 2003; Webster and Nichols, 2012; Norsworthy et al., 2014). *Amaranthus* species can also quickly develop resistance to herbicides, with several known populations in the U.S. resistant to five different herbicide modes of action (Webster and Nichols, 2012; Heap, 2022). Additionally, we found reductions in growth of *Echniochloa crus-galli*, *Abutilon theophrasti*, and *Ipomoea hederacea* in carbon-amended soils.

Not all species in this study were suppressed by microbial nitrogen immobilization. We found no evidence that *Ambrosia artemisiifolia* or *Trifolium repens* had any differences in plant growth per plant or per pot based on carbon amendment treatments. *Trifolium repens* is a legume with the ability to form a symbiosis with rhizobia bacteria that fix atmospheric nitrogen for plant uptake. The $^{15}$N data demonstrated that nitrogen fixation was the primary source of nitrogen in the carbon-amended treatment for *T. repens*. The data support the concept that legumes may be more successful in soils with high levels of nitrogen immobilization. *Ambrosia artemisiifolia* is a highly plastic annual species that can grow in high abiotic stress
conditions, such as in drought and salt-stressed soils (Onen et al., 2017). Research has also suggested that although *A. artemisiifolia* increases growth with increased nitrogen availability when grown in monoculture, it is a poor competitor with other plant species in nitrogen-rich environments (Leskovsek et al., 2012). The other plant species in our study, *Chenopodium album*, had less of a response to changing nitrogen availability, with reduced total growth at \( p=0.1 \) in carbon amended soil.

Most of the plant species in this study were impacted by carbon amendment through reductions in soil nitrogen availability, which can be used as a strategic weed management tool. In an integrated weed management approach, a variety of techniques are used together to manage weeds, ideally responding to growth traits of the predominant weeds in a field (Liebman et al. 2001). We found that it is possible to increase microbial growth and stimulate soil nitrogen immobilization through high carbon amendments (Chen et al., 2011; Kallenbach and Grandy; 2011). This strategy may be most useful in a field dominated by highly nitrogen-responsive weeds, such as *Amaranthus* species, but not in a field dominated by a stress-tolerant weed such as *A. artemisiifolia* (Grime, 1977).

2.5.2 Carbon amendments altered soil resource availability

With the addition of high carbon soil amendments, there was a subsequent change in carbon and nitrogen availability resulting in microbial nitrogen immobilization. In carbon amended pots, we saw increased soil C:N and increased microbial growth measured by higher microbial biomass carbon and nitrogen. There was also lower available nitrogen throughout the growing season, which was measured by extracting nitrate, the most plant-accessible form of nitrogen, from ion-exchange
resin bags (Fig. 2.2). Carbon amended pots were associated with higher soil C:N, and higher microbial biomass carbon and nitrogen, and unamended pots were associated with higher nitrate availability. These results are consistent with previous studies in which high rates of carbon amendments were associated with increased levels of soil carbon (Baer et al., 2003; Prober et al., 2005; Knauf et al., 2021), increases in microbial biomass (Baer et al., 2003; Eschen et al., 2007), and decreases in plant-available nitrogen (McLendon and Redente, 1992; Baer et al., 2003; Blumenthal et al., 2003; Prober et al., 2005; Eschen et al., 2007).

Measurements of $\delta^{15}$N in the aboveground plant tissues provided additional evidence of slower nitrogen cycling in the carbon-amended treatment relative to the unamended soils. For this analysis, we used aboveground plant tissue $\delta^{15}$N as a phytometer to estimate the nitrogen isotope ratio of the soil solution. Nitrogen transformation processes occurring in soil (most notably nitrification and denitrification) tend to deplete the soil solution and enrich the remaining soil nitrogen because both are fractionating processes (Driscoll and Ehleringer, 2021). Therefore, plants growing in a soil with more rapid nitrogen cycling will exhibit a more positive $\delta^{15}$N as we see in the unamended soils in this study. In addition, plants grown in carbon-amended soils had consistently lower $\delta^{15}$N than plants grown in unamended soils. The reduction indicates slower nitrogen mineralization in these pots due to microbial nitrogen immobilization. The one exception in this study was *Trifolium repens*, the leguminous white clover, in carbon-amended soil. In this case, $\delta^{15}$N was similar between plant material and the air, indicating that the primary nitrogen source for *T. repens* was nitrogen fixation in the carbon-amended treatment. This finding implies that *T. repens*
relied almost exclusively on nitrogen fixation when growing in carbon-amended soils, but relied on soil nitrogen in unamended soils. Nitrogen fixation decreases in high soil nitrogen environments (Salvagiotti et al., 2008), so this finding represents more evidence that we successfully decreased plant-available nitrogen with our carbon amendments.

2.5.3 Changing resource availability altered microbial community structure and function

Aside from increasing microbial community growth, carbon amendments also consistently changed microbial community composition. Bacterial and fungal beta diversity were significantly altered by carbon amendment. Both bacterial and fungal communities from soils amended with carbon were more similar to each other than to communities from unamended soils. Some of these community composition shifts were associated with differences in within-sample (alpha) diversity. Carbon-amended and unamended soils had similar bacterial diversity metrics (richness, evenness, and Shannon diversity), with the exception of higher Shannon diversity in carbon-amended soils growing *T. repens*. Since *T. repens* is a legume and supports the growth of nitrogen-fixing rhizobia, especially in nitrogen-limited environments, this result is not unexpected. However, soil fungal communities had lower diversity metrics (evenness, richness, and Shannon diversity) in carbon amended soils as compared to unamended soils. It is unclear why fungal and bacterial communities responded differently to the same resource changes, which could be due to their different physiologies. Bacteria have faster growth rates with an average C:N between 3 and 6, and fungi have slower growth rates with an average C:N between 5 and 15 (McGill and Cole, 1981). As a group, fungi
have more enzymes than bacteria that code for the breakdown of complex carbon compounds found in many high carbon amendments (de Boer et al., 2005). These differences enable fungi to be more successful (Strickland and Rousk, 2010) and often lead to an increase in relative abundance of fungal communities (Malik et al., 2016) in high carbon soils. We did not measure relative abundance of fungi compared to bacteria in our study, but fungi may have been more affected by high-carbon amendments relative to bacteria.

The decrease in diversity of fungal taxa in carbon-amended soils, especially coupled with an increase in abundance of OTUs that most accurately predict soil treatment in carbon-amended soils, may indicate that sawdust additions to the soil stimulated wood-decomposing specialist growth and establishment. In plant and sometimes animal communities, increasing resource availability often leads to greater species diversity as resource constraints are lifted (Mittelbach et al., 2001). After a threshold is reached, diversity then begins to decrease as specialists in utilizing the added resource begin to dominate. Thus, there is often a unimodal (hump-shaped) relationship between productivity or growth and diversity (Grime, 1973; Mittelbach et al. 2001). Several studies have observed an increasing or unimodal response in bacterial diversity with increased carbon resources (Delgado-Baquerizo, 2016; Geyer and Barrett, 2019; Bastida et al., 2021). But few studies have focused on changes in fungal community diversity. Ni et al. (2018) found that C:N was negatively correlated with fungal richness. Bastida et al. (2021) found increasing richness but decreasing richness:biomass as soil carbon increased. It is possible that the high amount of carbon added to the soil in our study may have passed peak diversity and begun to select for
fungal specialists. Most of the fungal OTUs in our study identified by Random Forest and Maaslin2 were enriched in carbon-amended pots, and absent or present at low levels in unamended pots. These OTUs include multiple carbon- or wood-decomposers and may be further evidence of selection for carbon-decomposing specialists. Of the 15 fungal OTUs identified by both algorithms, six were Basidiomycota, which evolved with the ability to mineralize lignocellulose and gain energy from the carbon trapped in plant litter and biomass (Schmidt-Dannert, 2016). Two were within the genus Subulicystidium, which are corticioid fungi with long- or short- wind-carried spores, and fruiting bodies often found on the underside of logs (Ordynets et al., 2018). One Basidiomycota was identified as Leucosporidium drummii, a saprotrophic dimorphic fungi, primarily found in decaying plant material, with low incidence in soil (Yurkov et al., 2012). Another Basidiomycota was identified to the order Sebacinales, which is a highly diverse order, including many endophytic and mycorrhizal fungi that range in relationship with plant roots from mildly negative to beneficial (Weiss et al., 2016). Less is known about Sebacinales living in bulk soil, however some may be able to live in high organic matter soil (Weiss et al., 2016).

Four fungal Ascomycetes were also identified. Acanthostigma filiforme is usually found on the living bark of oaks (Sanchez et al., 2012), decaying wood, and Fraxinus americana (Reblova and Barr, 2000). An unidentified species in the genus Podospora, which are typically found in almost all herbivore dung, was not wood-associated (Melo et al., 2015). Botryotrichum atrogriseum, which is also found in both soil and herbivore dung and is not wood-associated (Wang et al., 2016), was more
abundant in unamended soil. Of the other Random Forest and Maaslin2 identified fungi, none were identifiable.

Bacteria may have shown greater responses to nutrient limitation of nitrogen or phosphorous instead of carbon (Delgado-Baquerizo et al., 2017). Of the 12 bacterial OTUs identified by both Random Forest and Maaslin2, none were identifiable to the species level. The lack of information stemming from low resolution taxonomic profiling makes bacterial sequence data difficult to interpret in this study. Five of the identified OTUs were in the genus *BD2-6*, which is associated with high carbon environments, fermentation, and nitrogen cycling (Roy et al., 2018). However, one of those five was more abundant in unamended soil. One OTU was identified to the genus *Helicobacter*, which is typically an enteric or gastric bacteria (Gueneau and Loiseaux-De Goer, 2002) not generally found in soil (Kawaguchi et al., 2009). Three OTUs were identified to the family *Rhodobacteraceae*, which is usually associated with oligotrophic marine-aquatic environments and may be involved in biofilm formation but has versatile physiology and is also found in non-marine environments (Elifantz et al., 2013; Simon et al., 2017). One of these, *Rhodobacteraceae* was more abundant in unamended soil.

2.5.4 Conclusion

Our results indicate that soil resource modification can be a useful method to alter microbial community structure and function resulting in microbial immobilization of soil nitrogen and weed growth suppression. Consistent with our predictions, high-carbon amendments stimulated microbial growth and immobilized nitrogen. Plant species responded differently to this change in resource availability, and nitrophilous
plant species were negatively affected. Several nitrophilous plant species are especially problematic agronomic weeds in the U.S., and this strategy may be an effective ecological-based weed management tool. Changing the resource availability of the soil also altered soil microbial community composition. Understanding how this community changed may help us to cultivate the development of weed-suppressive soils. Bacterial and fungal communities responded differently to carbon amendments. Bacterial communities had little change in diversity, while fungal communities showed decreased diversity but higher abundance of specific species, particularly wood-associated taxa. The different responses may highlight the contrasting nutrient requirements of bacteria and fungi and may indicate that soil fungal communities can show a unimodal response to increased carbon-resource availability. However, more work in this area is needed, especially in understanding the function of specific soil taxa. Greater understanding would allow the development and implementation of ecologically based strategies (i.e., nitrogen immobilization) to effectively manage weed populations in cropping systems.
REFERENCES


https://doi.org/10.1016/j.agee.2011.08.020


https://www.jstor.org/stable/4045456


https://doi.org/10.1007/s10340-012-0433-2


resources. Frontiers in Microbiology, 5, 22. https://doi.org/10.3389%2Ffmicb.2014.00022


CHAPTER 3

MICROBIAL NITROGEN IMMOBILIZATION REDUCES COMPETITIVE ADVANTAGE OF NITROPHILOUS PLANTS IN LEGUME SYSTEMS

3.1 Abstract

One way to alter the competitive advantage of plants is through changing resource availability. Plant species that are more competitive in high nitrogen soils may have greater reductions in growth in low resource soils, and plants with greater investment in belowground growth may then become more competitive. A useful system to test this hypothesis is in row-crop production, where high resource inputs have selected for problematic weed species that are highly responsive to nitrogen. We tested the ability of soil nitrogen immobilization to reduce the competitive advantage of nitrophilous weeds over nitrogen-fixing soybeans (Glycine max). This experiment was conducted in a greenhouse with two soybean varieties, a nodulating and a non-nodulating variety with the same lineage, and three common nitrophilous weeds: Palmer amaranth (Amaranthus palmeri S. Watson), Powell amaranth (Amaranthus powellii S. Watson), and common lambsquarters (Chenopodium album L.). The plants were grown in monoculture and in polyculture in carbon amended and unamended soils. We measured soil microbial growth and community composition, soil nitrogen availability, and plant growth after 7.5 weeks. Weed species biomass and competitive advantage was lower in carbon amended soils than in unamended soils, but soybean growth was unaffected. In carbon amended soils we observed nitrogen immobilization processes occurring with increased microbial biomass nitrogen and reduced plant-available nitrogen. These trends were mirrored in the microbial community, which had a
decreased abundance of nitrifying bacteria, an increased abundance of bacteria with low nutrient requirements, and a high potential ability to degrade compounds. There were also differences in belowground bio-geo-chemical cycling based on plant polyculture compared to plant monoculture competition, which may have impacted plant competition

3.2 Introduction

Plants can have competitive advantage due to traits that make them especially good at capturing resources, what is known as “competitive effect”, or due to traits that make plants especially good at tolerating low resource conditions, “competitive response” (Gioria and Osborne, 2014; Goldberg, 1990). Some of the traits that are often associated with high competitive effect include better ability to capture light, high dispersal ability, high leaf:shoot ratios, and high photosynthetic capacity (Booth et al., 2003; Tilman, 1985). Traits that can confer high competitive response include better ability to use scarce abiotic resources, more belowground investment, and higher root:shoot ratios (Booth et al., 2003; Tilman, 1985). It may be possible to reduce a plant’s competitive advantage by reducing resource availability if the advantage is conferred through high competitive effect. One way to reduce soil nitrogen availability is through nitrogen immobilization. Nitrogen is immobilized when soil microbial growth is stimulated through carbon amendments, which then causes microbial nitrogen assimilation to maintain microbial stoichiometric ratios (Cleveland and Liptzin, 2007; Kallenbach and Grandy, 2011; Mooshammer et al., 2014).

Higher nitrogen availability may be the cause of increased plant invasions (Davis et al. 2000; Vitousek and White 1991), and so studies reducing soil available
nitrogen have been done in an attempt to restore native plant species growth (Blumenthal et al., 2003; Knauf et al. 2021; McLendon and Redente, 1992). These studies have been met with some success, however one limitation of these studies is that outside of the greenhouse they have not been able to incorporate carbon amendments into the soil, instead leaving them on the soil surface, which can reduce the extent of nitrogen immobilization. Agricultural systems may be a more useful setting to study the effect of nitrogen immobilization on plant community response since they are highly managed. Several of the most economically challenging weed species in the United States (U.S.) have a high competitive advantage, most likely from a high competitive effect. Many Amaranthus species are very successful competitors for light, water, and nitrogen (nitrophilous) (Blackshaw et al., 2003; Costea et al., 2004; Lindsey et al., 2013; Moreau et al., 2014). They also have the ability to substantially reduce yield, and are ranked as one of the most troublesome weeds in the U.S. (Roberts and Florentine, 2021; Van Wychen, 2021). Soybeans, on the other hand, can fix nitrogen from the atmosphere (Mourtzinis et al., 2018) and may have a stronger competitive response (van Heemst, 1985). Reducing the amount of soil available nitrogen may reduce the competitive ability of Amaranthus spp. in relation to soybeans by shifting the relative importance in competitive ability from high competitive effect to high competitive response (Goldberg, 1990; Suding et al., 2004).

Soybeans (Glycine max) are an economically important crop in the United States (U.S.), which is one of the largest exporters of soybeans in the world (Taheripour and Tyner 2018). Weed competition is a serious concern for soybean producers, and without control measures, yield loss is estimated to range from 10% to over 50% (Soltani et al.,
2017; Staniforth and Weber, 1956). However, the introduction of roundup-ready soybean varieties in 1996 dramatically simplified weed control in soybean fields, enabling growers to rely almost exclusively on glyphosate for weed management. Since this introduction, global glyphosate use increased 15-fold by 2014, and the percent of soybean acres in the U.S. treated with glyphosate rose to over 95% (Benbrook, 2016). However, with such heavy use of glyphosate, many weeds, including many species of Amaranthus, have evolved resistance (Heap and Duke, 2018). Shifting soil resource availability may be able to reduce the competitive advantage of nitrophilous weed species over soybeans, lessening the negative impact of plant competition on crop production.

We wanted to test the hypothesis that soil carbon amendments could alter the microbial community and cause nitrogen immobilization and consequently reduce the competitive ability of nitrophilous plants in relation to nitrogen-fixing soybeans. We amended soils with a mixture of sawdust and sugar as a high carbon source, or left soils unamended. We predicted that the microbial community would have increased growth but slowed nitrogen cycling in carbon amended soils, reducing plant-available nitrogen. We included three economically important weed species: two from the genus Amaranthus: Palmer amaranth (Amaranthus palmeri S. Watson) and Powell amaranth (Amaranthus powellii S. Watson), and one additional nitrogen-responsive weed: common lambsquarters (Chenopodium album L.). These weeds were grown in competition with two soybean varieties, one that nodulates, and one with reduced nodulation, grown in carbon amended and unamended soils in a greenhouse. We predicted that all three weed species would have a higher competitive advantage than
both soybean varieties in unamended soil. However, in carbon amended soil, we predicted that the competitive advantage of weeds would decrease, but that the nodulating soybean variety would rely more on nitrogen fixation as its nitrogen source and be less affected. We predicted that the non-nodulating soybean variety would have a similar reduction in growth to the weed species since it would not be able to rely on atmospheric nitrogen fixation.

3.3 Material and methods

3.3.1 Greenhouse set-up

To test how carbon amended soil affects soybean-weed competition, two soybean cultivars and three weed species were grown in a greenhouse study. The two soybean cultivars were: BARC-15 nodulated (strain 602449, maturity group IV), and BARC-15 non-nodulated (strain 602450, maturity group IV) (United States Department of Agriculture, Soybean Germplasm Collection, Urbana, IL). Both cultivars had the same pedigree (CX797-21(4) × Clark rj1) however, strain 602449 nodulated and 602450 did not nodulate. The weed species included were: A. palmeri, A. powellii, and C. album. Both A. powellii and C. album were grown from seed locally collected within 50 miles of Ithaca, NY, and A. palmeri seed was collected from a farm in Seneca County, NY.

Two soil amendment treatments were applied: carbon amended and unamended soils. Pots 15 cm in diameter and 14 cm deep were filled with a 2:1:1:1 mix of 2-mm sieved field soil, sand, Cornell mix, and perlite. Field soil was collected from the Caldwell Field Research Complex in Ithaca, NY (42°27’02.3” N, 76°27’41.1” W). The soil is classified as Williamson very fine sandy loam (coarse-silty, mixed, active, mesic
Typic Fragiudept). Half the pots were amended with 27.7 g dry weight sawdust and 1 g of sucrose. Sawdust was 48% carbon and sucrose was 42% carbon, for a total of 13.8 g of additional carbon per carbon amended pot.

Each pot had two plants growing in monoculture (intraspecific competition), or in polyculture (competition with a different species or cultivar). The experiment was not a full factorial design, weed species were only planted in interspecific competition with the soybeans and not in interspecific competition with other weeds. This totaled 12 plant combinations, with two different treatments, replicated 6 times each, for a total of 144 pots (Fig. 3.1). After seeding, each pot was inoculated with 1 tsp of rhizobia (NDure Soybean, Verdesian, Cary, NC). Plants were grown with a 15/9 light/dark photoperiod at 23°C. Pots were randomized once a week to reduce the effect of microclimate variation. Missing plants, many of which had been eaten by seedcorn maggot [Delia platura (Meigen)], were re-seeded two weeks after the original seeding date and there was no significant effect of reseeded plants (data not shown). The greenhouse temperature was increased to 27°C for one week to improve germination after re-seeding, and then returned to 23°C. Pots were watered as needed and were not fertilized. Plants were harvested 53 days after seeding.
Figure 3.1. Representation of experimental design. Black pots were carbon amended and grey pots were unamended. Plant monoculture combinations in teal included: *Amaranthus palmeri* (W₁), *Amaranthus powellii* (W₂), *Chenopodium album* (W₃), nodulating soybean variety (S₁), and non-nodulating soybean variety (S₂). Plant polyculture combinations in white included: S₁ x S₂, W₁ x S₁, W₂ x S₁, W₃ x S₁, W₁ x S₂, W₂ x S₂, and W₃ x S₂. These 24 pot combinations were replicated 6 times for a total of 144 pots.
3.3.2 Plant analyses

At harvest, plants were cut at the soil surface and the aboveground biomass of each plant was dried at 60°C and weighed individually. Plant roots were carefully extracted from the soil and stored at 3°C until they were rinsed, dried, and weighed. Photos were taken of all soybean roots before drying and the number of root nodules in each photo was counted.

3.3.3 Soil analyses

At plant harvest, a subset of fresh soil was stored at 3°C until processed for nitrate and ammonium analysis, microbial biomass, soil moisture, and pH. An aliquot of fresh soil was also frozen within three days of harvest for DNA amplification. Nitrate and ammonium were measured using a 2 M KCl extraction from about 8 g of fresh soil, shaken for 2 hours at 180 rpm (reciprocal shaker, E6000 Medium-Duty; Eberbach Corporation, Ann Arbor, MI), and filtered through Whatman 1 filter paper. Filtrate was stored frozen at -20°C until analysis. Filtrate was analyzed for nitrate and ammonium following the method by Hood-Nowotny et al. (2010). Absorbance was read at 540 nm and 660 nm on a 96-well microplate reader for nitrate and ammonium, respectively (Synergy HT; Bio-Tek Instruments, Winooski, VT). Microbial biomass was measured using a fumigation extraction method. The difference between carbon and nitrogen extracted from fumigated soil and extracted from unfumigated soil are a proxy for microbial biomass carbon and nitrogen. These proxies were divided by 0.45 (carbon) and 0.54 (nitrogen) as correction factors to get a true microbial biomass estimate (Joergensen and Mueller, 1996; Vance et al., 1987). Soil was fumigated using a vacuum pump to vaporize CHCl₃ within a desiccator for 20 hours. To extract, about 8 g of soil
was shaken at 180 rpm for 2 hours with 0.05 M K$_2$SO$_4$ before filtering through Whatman 1 filter paper. Filtrate was collected and stored frozen at -20°C until analysis. Total nitrogen, inorganic carbon, and organic carbon was measured on a TOC/TN analyzer (TOC/TN Analyzer; Shimadzu Scientific Instruments, Columbia, MD). Soil moisture was calculated by weighing a 10 g subsample of fresh soil into a tin cup and drying at 105°C in an oven (Isotemp Oven; Fisher Scientific, Suwanee, GA) for 24 hours before reweighing. To measure pH, 3.0 ± 0.1 g of dried soil was mixed with 6 mL of deionized water in a 15 mL falcon tube and shaken on a reciprocal shaker (E6000 Medium-Duty; Eberbach Corporation, Ann Arbor, MI) at 180 revolutions per minute for 60 minutes. Tubes were removed from the shaker and allowed to sit for at least 10 minutes before pH measurements were taken with a pH meter (Orion Star A215, Thermo Scientific, Waltham, MA). Soil carbon and nitrogen were measured using soil that had been air-dried and ground into a fine powder using a mortar and pestle, rinsing with ethanol between samples. Soil was submitted to the Cornell Stable Isotope Lab for total C, N, and δ$^{15}$N analysis on an isotope ratio mass spectrometer (Delta V, Thermo Scientific, Waltham, MA) connected to an elemental analyzer (NC2500, Thermo Scientific, Waltham, MA).

3.3.4 Microbial amplification

We assessed bacterial and fungal community composition with high-throughput amplicon sequencing. DNA was extracted from soil according to the protocol detailed in the Qiagen DNeasy soil DNA extraction kit (Beverly, MA), using approximately 200 mg of soil. Sequencing targeted the V3-V4 region of the 16S rRNA gene (16S) in bacteria and the ITS2 region of the fungal internal transcribed spacer gene (ITS) in
fungi. The 341F and 805R (Herlemann et al., 2011) and ITS1F and 58A2R (Gardes and Bruns, 1993; Martin and Rygiewicz, 2005) primers were used to isolate the 16S and ITS regions, respectively. Samples were amplified on a Bio-Rad C1000 Thermal Cycler (Hercules, CA, USA). Amplification settings for bacteria were: 2 minutes at 94°C, then 25 cycles of 20 seconds at 94°C, 20 seconds at 55°C, and 30 seconds at 72°C, followed by a 3 minute elongation at 72°C. Amplification settings for fungi were: 3 minutes at 94°C, then 40 cycles of 20 seconds at 94°C, 30 seconds at 45°C, and 45 seconds at 72°C, followed by a 5 minute elongation at 72°C. Amplified samples were cleaned using the protocol detailed in MagBio HighPrep PCR beads (MagBio Genomics, Gaithersburg, MD, USA). Nextera XT prep index primers (Illumina, San Diego, CA, USA) were attached. Barcoded amplicons were normalized using the protocol detailed in the SequalPrep Normalization Kit (Thermo Fisher Scientific, Waltham, MA. USA). Samples were pooled and concentrated using a Centri-vap DNA concentrator (Labconco, Kansas City, MO, USA). Concentrated samples underwent a final cleaning by running them through a 1.2% gel, excising the bands of expected size, and processing using the protocol detailed in Wizard SVG gel and PCR clean-up system (Promega, Madison, WI, USA). Samples were sequenced on the Illumina MiSeq at the Cornell Genomics Facility (Ithaca, NY) using a 600-cycle MiSeq Reagent Kit v.3 for the 16S pool and a 500-cycle MiSeq Reagent Kit v.2 for the ITS pool.

Raw sequence reads were merged and filtered in Qiime2 (Bolyen et al., 2019). To demultiplex and merge paired ends, q2-demux was used. Sequences were denoised and amplicon sequence variants (ASVs) were identified using DADA2. To cluster ASVs and create a table of assigned operational taxonomic units (OTUs), de-novo q2-
vsearch was used, with a 97% shared identity as the cutoff. To create a feature table, q2-feature-table was run on the OTU table. Bacterial and fungal classifiers were trained with a Naïve Bayes classifier trained on the 99% Silva 138 database for bacteria (Bokulich et al., 2018; Robeson et al., 2020) and the UNITE 13.8 database for fungi (Abarenkov et al., 2010). Classify-sklearn was used to assign taxonomy to sequences.

3.3.5 Statistical analysis

All statistics were completed in R (version 4.1.3) (R Core Team, 2022). Soil response was analyzed with a linear model (function `lm`, in package stats, version 3.6.2) with soil amendment treatment and the plant combinations growing in the pot plus their interaction as independent variables. A square root transformation was done on any data that did not satisfy assumptions of normality, although all data presented in the results are back transformed. A type III analysis of variance (ANOVA) (function `anova`, in package stats, version 3.6.2) was run on the linear model to test for significant effects. Post-hoc analysis for all models was done using estimated model means (function `emmeans`, package emmeans, version 0.9) with Tukey’s correction for multiple means comparisons (Lenth, 2022).

To analyze soybean and weed growth response, data were reorganized into a long format so that aboveground biomass, root biomass, and the root:shoot ratio for each plant could be tested individually. The effects of soil amendment, plant type, plant combination growing in the pot, and the interactions between amendment and plant type and amendment and plant combination were the independent variables analyzed with a type III ANOVA (function `anova`, in package stats, version 3.6.2) using Kenward-Roger denominator degrees of freedom, run on a linear mixed model (function `lmer`, in
package lme4, version 1.1-31) (Bates et al., 2015). Individual pots were included as a random effect, although there was no measurable effect of pot. Nodulation was analyzed similarly, including only pots that grew soybeans in the analysis.

Plant competition was analyzed by first calculating an average aboveground plant biomass when grown in monoculture in both carbon amended and unamended soils. The difference between the average biomass in monoculture and the aboveground biomass of each plant grown in polyculture in either carbon amended or unamended soils was calculated. This difference is the relative competitive ability of each plant in amended and unamended soils. The change in biomass was analyzed using the same linear mixed model for plant growth metrics as described above.

Soil microbial abundance data were prepared for analysis by first removing samples with fewer than 1,000 (bacterial) or 2,000 (fungal) reads. Remaining read counts were converted to percent abundance and then into a Bray-Curtis distance matrix (function vegdist, in package vegan, version 2.5-7) (Dixon, 2003). Beta diversity was analyzed using a principal coordinate analysis (PCoA) (function cmdscale, in package stats, version 4.1.2) of the distance matrices. Treatment effects and pseudo-F-ratios were estimated using a Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations (function adonis2, in package vegan, version 2.5-7). Alpha diversity was estimated with a Shannon diversity index (function diversity, in package vegan, version 2.5-7), OTU counts for richness, and a Pilou evenness index (function diversity, in package vegan, version 2.5-7). Diversity indices were analyzed using the same linear model for soil traits as described above.
Bacterial pathway abundance was estimated using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST2) qiime2 plugin (Douglas et al., 2020). Estimated pathway abundance data were uploaded to MetaCyc where they were classified by pathway type ontology (Caspi et al., 2014). Ontology data were downloaded and the most specific pathway type (right-most) was set for each pathway. The classification “super pathways” was only used if no other pathway types were known (and renamed “unclassified super pathway” for downstream analysis). Pathway types were attached to estimated pathway abundance data and estimated pathway counts were summed by pathway type. Pathway types associated with carbon amended or unamended soils were found using the function Microbiome Multivariable Association with Linear Models 2.0 (Maaslin2) (function Maaslin2, in package Maaslin2, version 1.8.0) (Mallick et al., 2021). The top 10 most differentially abundant pathways were further analyzed for patterns based on soil amended treatment.

3.4 Results

3.4.1 Plant growth response and competition

We found evidence of uneven reductions in plant growth in carbon amended soils. The aboveground plant biomass (df=4, F-value=18.00, p<0.0001), root biomass (df=4, F-value=7.37, p<0.0001), and root:shoot (df=4, F-value=3.39, p=0.01) all had significant interactions between amendment treatment and the plant type (Table 3.1). Weed species growing in unamended pots had greater aboveground and root biomass than weed species grown in carbon amended pots (Fig. 3.2). However, the biomass of either soybean variety was not different based on soil amendment treatment. The trend was opposite for root:shoot ratios. Weed species growing in unamended pots had lower
root:shoot than weed species grown in carbon amended pots (Fig. 3.2). However, the root:shoot of either soybean variety was not different based on soil amendment treatment.

Table 3.1. P-values and F-values of aboveground biomass, root biomass, and root:shoot from a type III ANOVA of a linear model for each independent variable included in the model. Significant p-values (p<0.05) are bold.

<table>
<thead>
<tr>
<th>Variable</th>
<th>F value</th>
<th>P-value</th>
<th>F value</th>
<th>P-value</th>
<th>F value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aboveground biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amendment</td>
<td>380.40</td>
<td>&lt;0.0001</td>
<td>156.74</td>
<td>&lt;0.0001</td>
<td>82.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plant species</td>
<td>0.69</td>
<td>0.59</td>
<td>2.00</td>
<td>0.10</td>
<td>1.57</td>
<td>0.19</td>
</tr>
<tr>
<td>Neighbor plant (in pot)</td>
<td>1.47</td>
<td>0.15</td>
<td>1.33</td>
<td>0.21</td>
<td>0.78</td>
<td>0.66</td>
</tr>
<tr>
<td>Amendment × plant</td>
<td>18.00</td>
<td>&lt;0.0001</td>
<td>7.37</td>
<td>&lt;0.0001</td>
<td>3.39</td>
<td>0.01</td>
</tr>
<tr>
<td>Amendment × neighbor</td>
<td>1.69</td>
<td>0.08</td>
<td>1.60</td>
<td>0.10</td>
<td>0.51</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Figure 3.2. Mean plant biomass in pots amended with carbon or unamended. Bars labeled with different letters indicate significantly different means (p<0.05) by soil amendment treatment.
Plant competition was also affected differently based on soil carbon amendments. The interaction between carbon amendment treatment and plant type affected the change in aboveground biomass in polyculture from aboveground biomass in monoculture (df=2, F-value=12.11, p<0.0001). Both soybean varieties (nodulating and non-nodulating) had no difference in aboveground biomass growing in monoculture or in polyculture in carbon amended pots or unamended pots (Fig. 3.3). However, weed species had significantly higher aboveground biomass in polyculture than in monoculture in unamended pots only. In carbon amended pots, weed species had no difference in aboveground biomass in polyculture from monoculture.

Average root nodulation of the nodulating soybean variety (15.13 nodules per plant) was higher than the root nodulation of the non-nodulating soybean (8.14 nodules per plant) (df=1, F-value=93.60, p<0.0001). There was also a significant interaction between the soil amendment treatment and the plant (df=1, F-value=4.66, p=0.03) (Fig. 3.4) and the plant combination growing in the same pot (df=8, F-value=2.13, p=0.04). The nodulating soybean variety grown in competition with *Amaranthus powellii* had a higher nodulation rate (30.13 nodules per plant) than any of the non-nodulating soybeans or the nodulating soybean grown in competition with either the non-nodulating soybean or *Chenopodium album* (between 3.66 and 13.17 nodules per plant).
Figure 3.3. Mean change in biomass between plant varieties grown in polyculture and monoculture. Boxes labeled with different letters indicate significantly different means (p<0.05) by soil amendment treatment.
3.4.2 Soil characteristics

The soil pH (df=1, F-value=0.57, p=0.45), and microbial biomass carbon (df=1, F-value=1.32, p=0.25) were not different based on soil amendment treatment or any independent variable. However, microbial biomass nitrogen was higher in carbon amended pots (df=1, F-value=268.35, p<0.0001) (Fig. 3.5) and varied based on the plant combination growing in the pot (df=5, F-value=3.44, p=0.006), but was not affected by their interaction (df=5, F-value=0.92, p=0.47). Soil nitrate was lower in carbon amended pots (df=1, F-value=12.05, p=0.0007), but soil ammonium was higher in carbon amended pots (df=1, F-value=55.64, p<0.0001). Soil moisture was higher in carbon amended pots (df=1, F-value=183.48, p<0.0001) but also had a significant interaction
between carbon amendment and the plant combination growing in the pot (df=5, F-value=5.46, p=0.0001). Despite this significant interaction, every plant combination had higher soil moisture in carbon amended pots than unamended pots, except nodulating soybeans grown in monoculture, which had no difference in soil moisture based on soil amendment treatment (data not shown). Total soil C:N was higher in carbon amended pots (df=1, F-value=6.52, p=0.01) but also had a significant interaction between the soil amendment treatment and the plant combination growing in the pot (df=5, F-value=10.53, p<0.0001). When any of the plants were grown in monoculture, carbon amended soils had higher C:N (Fig. 3.6). However, when grown in polyculture, the soil C:N in pots with nodulating soybean grown in competition with any of the weed species was lower in carbon amended soils. Soil δ¹⁵N also had a significant interaction between soil amendment treatment and the plant combination growing in the pot (df=5, F-value=10.20, p<0.0001), but the trend was the opposite. When any of the plants were grown in polyculture, carbon amended soils had higher δ¹⁵N (Fig. 3.6). However, when grown in monoculture, the soil δ¹⁵N did not vary based on soil amendment treatment.
Figure 3.5. Mean soil traits in pots amended with carbon or unamended. Bars with ** indicate significantly different means (p<0.05) by soil amendment treatment.
Figure 3.6. Mean soil traits in pots amended with carbon or unamended growing plants in monoculture or in polyculture. Bars with ** indicate significantly different means (p<0.05) by soil amendment treatment. Mean percent soil carbon and percent soil nitrogen are listed in blue and red, respectively, in the soil C:N graph.
Table 3.2. Mean diversity index values and p-values for bacterial and fungal communities in soils amended with carbon, or unamended. Means are shown for each combination of plants growing in a pot (monocultures or polyculture). p-values are calculated from Tukey’s post-hoc analysis of a type III ANOVA of a linear model for diversity index. Significant p-values (p<0.05) are bold and indicate that means are different between carbon amended and unamended pots growing the corresponding plant combination.

<table>
<thead>
<tr>
<th>Pot combination</th>
<th>Shannon diversity (Bacteria)</th>
<th>Richness (Bacteria)</th>
<th>Evenness (Bacteria)</th>
<th>Shannon diversity (Fungi)</th>
<th>Richness (Fungi)</th>
<th>Evenness (Fungi)</th>
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<td>65.2</td>
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</tr>
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3.4.3 Soil microbial community structure and function

A total of 1,978 unique bacterial and 2,310 unique fungal sequences were observed, with a mean of 4,138 bacterial reads and 18,126 fungal reads per pot. Bacterial alpha and beta diversity were affected by the interaction between the soil amendment treatment and the plant combination growing in the pot. Shannon diversity (df=11, F-value=2.74, p=0.004), richness (df=11, F-value=5.90, p<0.0001), and evenness (df=11, F-value=2.69, p=0.004) were all affected by the interaction of these treatments (Table 3.2). The Principal Coordinates Analysis (PCoA) of bacterial sequences (Fig. 3.7a) and the corresponding PERMANOVA analysis also identified a difference in diversity for bacterial sequences based on the interaction between the soil amendment treatment and the plant combination growing in the pot (df=11, F Model=1.50, p=0.001). Fungal alpha and beta diversity were affected by the soil amendment treatment. Shannon diversity (df=1, F-value=301.54, p<0.0001) and richness (df=1, F-value=314.24, p<0.0001) were both higher in unamended pots than carbon amended pots. The PCoA of fungal sequences (Fig. 3.7b) and the corresponding PERMANOVA analysis showed a clear separation between unamended and carbon amended pots (df=1, F Model=,169.17 p=0.001).
Figure 3.7. Principal coordinates analysis (PCoA) of the bacterial (A) and fungal (B) communities based on Illumina MiSeq amplicon sequencing. Shape and ellipse line indicates soil amendment treatment. Color indicates the combination of plants growing in each pot. PERMANOVA revealed significant effects of soil amendment treatment for bacterial and fungal community composition ($p$-value $= 0.001$ for bacteria and fungi), and an interaction between pot combination and soil amendment treatment for bacterial community composition ($p$-value $= 0.005$).
Relative abundance of bacterial and fungal genera varied by soil amendment treatment. *Bacillus* and *Arthrobacter* bacterial genera were more abundant in carbon amended soils, and *Nitrospira* and *Kaistobacter* were more abundant in unamended soils (Fig. 3.8). The fungal genus *Cercophora* was more abundant in carbon amended soils, and *Fusarium*, *Neocosmospora*, *Chaetomium*, and *Botryotrichum* were more abundant in unamended soils (Fig. 3.9). *Preussia* was most abundant in carbon amended soils growing monocultures of either *Amaranthus* spp.

For potential function, PICRUSt2 analysis revealed that twenty-five metabolic pathway types were differentially abundant based on the soil amendment treatment by Maaslin2 (q-value<0.05). Four of the top 10 most significantly different pathway types were more abundant in carbon amended soils: unclassified super pathways, secondary metabolite degradation, amine degradation, and alcohol degradation (Fig. 3.10). Six of the top 10 most significantly different pathway types were more abundant in unamended soils: secondary metabolite biosynthesis, nucleotide biosynthesis, nucleic acid processing, carbohydrate biosynthesis, C1 compounds (pathways that utilize one carbon compounds), and aminoacyl tRNAs charging.
Figure 3.8. Percent abundance of bacterial genera found in soils amended with carbon or unamended and growing different combinations of two plants per pot. Any genera with a median percent abundance less than 1% are not shown. These 7 genera only make up a small percentage of the total bacterial community, so relative abundance is only shown to 50%.
Figure 3.9. Percent abundance of fungal genera found in soils amended with carbon or unamended and growing different combinations of two plants per pot. Any genera with a median percent abundance less than 1% are not shown.
Figure 3.10. The 10 bacterial estimated gene pathway types which were most significantly different between carbon amended and unamended soils. These pathways were estimated using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) and classified using MetaCyc. Differentially abundant pathways were identified with Multivariable Associations with Linear Models (Maaslin2). Identified estimated pathways are organized based on whether they were more abundant in carbon amended or unamended soils. C1 compounds represents pathways that utilize compounds containing one carbon, such as CO₂, CO, formaldehyde, and methanol. P-values for the differential abundance are written in orange.

3.5 Discussion

3.5.1 Plant species had a differential growth response to carbon amendments

Plant species in this experiment were selected because they were expected to have a differential response to nitrogen immobilizing soils. All three weed species included in our study have been found to have increased nitrogen assimilation with
higher rates of nitrogen availability (Blackshaw et al., 2003; Costea et al., 2004; Lindsey et al., 2013) and were expected to be more competitive in high nitrogen soils. Therefore, these weeds were predicted to be less competitive in low available nitrogen soils, which was confirmed in our study. All three species had lower aboveground and root biomass in carbon amended soils than in unamended soils. The root:shoot was also higher in carbon amended soils than in unamended soils, suggesting a shift in resource allocation to belowground growth in low nutrient environments (Booth et al., 2003; Grigulis et al., 2013; Tilman, 1985).

Both soybean varieties had no significant difference in aboveground or root biomass based on soil amendment treatment. There was also no difference in root:shoot based on soil amendment treatment. Since soybeans can source between 25% and 95% of their nitrogen from nitrogen fixation (Mourtzinis et al., 2018), we did not expect a significant change in biomass in nitrogen immobilizing soils. We did expect the non-nodulating soybean to have a similar response in low available nitrogen soils as weeds, since its ability to rely on nitrogen fixation is much reduced. There were significantly fewer root nodules on non-nodulating soybean roots in carbon amended soils compared to the nodulating soybean roots in unamended soils, however there was still nodulation on the non-nodulating soybean roots. This may account for the non-nodulating soybean responding more similarly to the nodulating soybean than to weeds in carbon amended soils.

These different growth responses to carbon amendments translated into changes in the competitive ability of weeds over soybeans based on soil amendment treatments. For both soybean varieties there was no change in the differences in biomass between
soybeans in monoculture or polyculture based on soil amendment treatment. However, weeds responded differently. In unamended soils weeds had higher biomass when grown in competition with soybeans (polyculture) than weeds when grown in competition with themselves (monoculture). In carbon amended soils, this difference went away, meaning there was no difference in weed biomass in polyculture from weed biomass in monoculture. This change indicates that weeds are more competitive than soybeans in nitrogen mineralizing soils, but they are not more competitive than soybeans in nitrogen immobilizing soils. Similar trends have been seen in other greenhouse studies looking at soil carbon amendments. Perry et al. found that in carbon amended soil, the native Carex hystericina Muhl. ex Willd was more competitive than the weedy Phalaris arundinacea L., but that this trend was reversed in nitrogen amended soil (2004). However, Knauf et al. found that resource exploitative species had a greater increase in leaf area and total biomass with increasing nutrient availability, but that there was no subsequent change in competition (2021).

3.5.2 Carbon amendments altered soil nitrogen availability

Changes in plant growth and competition were likely due to changes in soil nitrogen availability. We observed several indicators of nitrogen immobilization in carbon amended soil. Soil available nitrate was significantly reduced in carbon amended soils at the time of harvest and microbial biomass nitrogen was significantly higher at this same time, suggesting that nitrogen was immobilized in the microbial community biomass (Cleveland and Liptzin, 2007; Kallenbach and Grandy, 2011). These findings fit with other studies, which have consistently found nitrogen immobilization caused by soil carbon amendments (Pittman et al., 2020; van der Sloot et al., 2022; Vinten et al.,
There was also higher soil moisture in our carbon amended soils, which is consistent with work that shows carbon amendments can improve water holding capacity (Celik et al., 2004; Li et al., 2018).

These observations of nitrogen immobilization are also supported by changes in the soil microbial community composition. In carbon amended soils, we saw a lower abundance of *Nitrospira* and *Kaistobacter* compared to unamended soil. *Nitrospira* are aerobic chemolithoautotrophic nitrite-oxidizing bacteria and are critical for the process of nitrification and the formation of nitrate. Comammox *Nitrospira* can convert ammonia to nitrite and then nitrite to nitrate (Hu et al., 2021) and other *Nitrospira* often live in close association with ammonia-oxidizing bacteria or archaea, which first convert ammonia to nitrite (Daims and Wagner, 2018). With a low abundance of *Nitrospira* in carbon amended soils, nitrate may be less available and ammonium may accumulate. We observed both a reduction in soil nitrate and an increase in soil ammonium in our carbon amended soils, which fits with the lower relative abundance of *Nitrospira*.

In carbon amended soils, we also saw a higher relative abundance of species in the *Bacillus* and *Arthrobacter* genera. Both are common in soils (Jones and Keddie, 2006; Saxena et al., 2020) and many *Arthrobacter* are stress tolerant and able to survive in low moisture and low food environments. They have a low vitamin requirement and can use a variety of carbon substrates for growth, so *Arthrobacter* are commonly found in nutrient poor soils, which is consistent with our results (Jones and Keddie, 2006). *Bacillus* are known to contain many plant-growth-promoting bacterial species. Many species are antimicrobial, antiviral, immunosuppressive, antitumor, or cause the release of plant phytohormones that further protect the plant from disease (Sansinenea and
Ortiz, 2011; Saxena et al., 2020). *Bacillus* can also improve nutrient cycling (Canbolat et al., 2005), which would be necessary in soils where microbes are limited by nutrients other than carbon.

The potential metabolic pathways of the bacterial community are also consistent with what we may expect in carbon amended soils. In these carbon amended soils, bacterial degradation estimated pathways were predominant, but in unamended soils, synthesis estimated pathways were predominant. It is important to keep in mind that metabolic gene pathway estimates from PICRUSt2 are only estimates of potential function based on community composition. Although they can be correlated with true metabolic activity (Agrawal et al., 2019; Raes et al., 2021), they are still subject to significant bias (Narayan et al., 2020). With that in mind, microbial carbon use efficiency is known to vary depending on microbial community composition (Glassman et al., 2018; Nielsen et al., 2011) as well as carbon substrate type (Qiao et al., 2019). The change in predominant metabolic activity that is suggested by our data may be one reason for changes in carbon use efficiency based on substrate type and microbial community composition. In carbon amended soils where energy resources are high and nutrient resources may be limiting, the bacterial community may first be degrading the carbon substrates to access nutrients, resulting in priming (Fontaine et al., 2004; Pascault et al., 2013).

We also observed a consistent decrease in fungal diversity in carbon amended soils. This is consistent with other studies that have explored fungal response to soil carbon additions (Bastida et al., 2021; Ni et al., 2018). Previous work in our lab hypothesized that decreased fungal diversity in high carbon amended soils may be due
to the productivity-diversity hypothesis, where fungi specializing in carbon substrate decomposition dominate the soil fungal community (Chapter 2 of this dissertation). Bacterial diversity was not consistently changed by the soil amendment treatment, but fungal physiology is more carbon dependent than bacteria (de Boer et al., 2005; McGill and Cole, 1981; Strickland and Rousk, 2010), so it may fit that the fungi were more affected by the carbon amendment. However, we did not see a clear connection between the carbon amendment treatment and changes in the dominant fungal genera. In carbon amended soils, *Cercophora*, was the predominant fungal genus, which can cause leaf-spot disease in various plant species (Kimber et al., 2016). In unamended soils, *Fusarium*, and *Neocosmospora* (which some scientists believe should also be classified as *Fusarium* (Geiser et al., 2013; O’Donnell et al., 2020)), were the predominant fungal genera (Siegel-Hertz, 2018; Smith, 2007).

3.5.3 Plant competition changed belowground bio-geo-chemical cycling

Along with consistent evidence of nitrogen immobilization in carbon amended soils, we also observed consistent evidence of increased belowground activity in plants grown in polyculture competition compared to plants grown as monocultures. This was especially evident when nodulating soybeans were grown in competition with weeds, which is also where we saw differences in competition based on soil amendment treatment.

When plants were grown in monoculture, we saw several of our predicted trends based on soil amendment treatments. Since we added an amendment with a high C:N for our carbon amendment treatment, we expected to see a subsequently higher soil C:N compared to unamended soils. We also did not expect to see a difference in soil $\delta^{15}N$.
based on soil amendment treatments. In carbon amended pots growing monocultures, soil C:N increased and $\delta^{15}$N was not different from unamended pots. However, in polyculture pots, there was no significant difference between C:N based on soil amendment treatment except when nodulating soybeans were grown with weeds. Soybean-weed polyculture actually led to higher soil C:N in unamended soils than in carbon amended soils. Also, $\delta^{15}$N in carbon amended soils was higher than in unamended soils. These trends were further supported by changes in bacterial alpha and beta diversity, which were both affected by the interaction between soil amendment treatment and plant combination growing in the pot.

This evidence suggests that in polyculture pots, the belowground activity changed dramatically, leading to increased soil C:N and changes in nitrogen cycling compared to unamended soils growing monocultures. There are many different reasons for changes in belowground activity and nutrient cycling including: greater plant nutrient uptake, greater root exudation, increased nitrogen fixation by plant-associated or free-living rhizobia, and changes in nitrogen fractionation and nitrification rates. We do not have the data to tease apart the effects of these different biological processes, however there is evidence from the literature that supports changes in soil bio-geo-chemical cycling based on plant diversity, competition, and their cascading effects on the rhizosphere (Wang et al., 2022).

Plant roots are well known to release root exudates, which can account for up to 80% of plant-fixed carbon (Rasmann and Turlings, 2016). These exudates facilitate the plant’s interaction with the belowground ecosystem: stimulating mutualistic as well as antagonistic exchanges with microbial communities, soil insects, and other plants
(Callaway, 2002; Li et al., 2016; Pierik et al., 2013; Rasmann and Turlings, 2016). It is estimated that there are thousands of different compounds exuded, depending on the plant species and the environment it is growing in, which results in highly complex signals that are difficult to tease apart (Pierik et al., 2013). Many plant species in polyculture produce plant-antagonistic root exudates and allelopathic compounds to improve their competitive ability against other plant species, but do not produce these compounds in monoculture (Hauggaard-Nielsen et al., 2001; Hazrati et al. 2020; He et al., 2013; Li et al., 1999; Li et al., 2016).

Since plant diversity often leads to an increased diversity of plant root exudates, bacterial diversity can have a corresponding response. Different root exudates can cause shifts in the microbial community (Costa et al., 2006), so it can follow that soil microbial communities increase in diversity with the diverse root exudate supply. There is some evidence that increased plant diversity through crop rotations leads to increased soil bacterial diversity (Berg and Smalla, 2009; Venter et al., 2016). However, other studies have also found that although plant community does play a role in shaping the microbial community composition, abiotic soil factors are often more influential (Nunan et al., 2005; Singh et al., 2009; Zhang et al., 2008).

These changes in plant and microbial community activity caused by plant competition may be playing a role in the changes in rhizosphere bio-geo-chemical cycling that we see in our study. In unamended soils growing polycultures, belowground activity such as root exudation, nitrogen fixation, nitrogen uptake, and microbial metabolite production are high. Nitrogen-responsive weeds are more competitive than soybeans in these relatively high resource soils. However, in carbon amended soils
growing polycultures, that belowground activity may be dramatically altered. The growth-differentiation balance framework suggests that synthesizing any plant defense mechanism comes at a metabolic cost to plant growth (Herms and Mattson, 1992; Fernandez et al., 2016). Therefore, it is not cost effective to produce secondary metabolites such as root exudates and allelochemicals in very high competition or low resource environments. This principle is often observed in root exudation patterns, with the highest levels of root exudation at intermediate levels of competition or stress (Fernandez et al., 2016; Kong et al., 2018; Li et al., 2016; Sugiyama et al., 2016). Soils in our study amended with carbon may have created high plant stress environments, where plants did not allocate resources to secondary metabolite production due to low nitrogen availability. Nitrogen responsive weeds were not competitive in that environment, and soybean growth remained unaffected.

3.5.4 Conclusion

In summary, we did see evidence that carbon amendments can reduce the growth and competitive advantage of some nitrophilous weeds by changing the soil nitrogen availability. These changes are reflected in the soil chemical composition, microbial community composition, and potential microbial community function. There were also differences in plant polyculture compared to plant monoculture competition with cascading effects on belowground bio-geo-chemistry. These effects were visible through differences in soil carbon and nitrogen cycling and may be related to the changes in weed competitive advantage. This work has important implications for the potential to use nitrogen immobilization as a weed management tool to reduce nitrophilous weed competitive advantage in soybean cropping systems.
REFERENCES

https://doi.org/10.1111/j.1469-8137.2009.03160.x

https://doi.org/10.1016/j.bej.2019.107328


https://doi.org/10.18637/jss.v067.i01

https://doi.org/10.1186/s12302-016-0070-0


Booth, B.D., Murphy, S.D., Swanton, C.J., 2003. Interactions between populations I: Competition and allelopathy. In B.D. Booth, S.D. Murphy, C.J. Swanton (Eds.) Weed ecology in natural and agricultural systems (pp.121). CABI.

Braz, G.B.P., Takano, H.K., 2022. Chemical control of multiple herbicide-resistant


https://doi.org/10.51694/AdvWeedSci/2022;40:Amaranthus009


Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G., Smalla, K., 2006. Effects of site and plant species on rhizosphere community structure as revealed by


https://doi.org/10.1111/j.1461-0248.2004.00579.x


Hazrati, H., Fomsgaard, I.S., Kudsk, P., 2021. Targeted metabolomics unveil alteration in accumulation and root exudation of flavonoids as a response to

https://doi.org/10.1080/17429145.2021.1881176


https://doi.org/10.1016/j.soilbio.2012.07.027


https://doi.org/10.1038/ismej.2011.41


composition and dynamics from DADA2-corrected 16S rDNA sequences.


https://doi.org/10.3389/fmicb.2018.01815


https://doi.org/10.1111/j.1365-2389.2010.01314.x


Continued Inclusion of the Fusarium solani Species Complex in the Genus Fusarium. mSphere 5, e00810-20. https://doi.org/10.1128/mSphere.00810-20


https://doi.org/10.1038/s41467-021-22409-4


https://doi.org/10.1016/j.crope.2022.08.001


https://doi.org/10.1016/0308-521X(85)90047-2

Van Wychen, L., 2021. WSSA survey ranks most common and most troublesome weeds in grass crops, pasture and turf [WWW Document].


https://doi.org/10.1016/j.pedobi.2016.04.001


https://doi.org/10.1007/s00374-002-0524-y


CHAPTER 4

SOIL AMENDMENTS WITH A HIGH C:N CAN IMPROVE SOIL BIOLOGICAL HEALTH METRICS AND MAY BE A USEFUL TOOL TO REDUCE WEED GROWTH IN SOYBEANS

4.1 Abstract

Nitrogen availability is an important mechanism in controlling agricultural weed growth since many weeds in annual cropping systems are more competitive in high nitrogen soils. A potential way to control nitrogen availability is through soil carbon amendments, which stimulate soil microbial growth and immobilize nitrogen. Carbon amendments can also increase soil biological functioning and improve soil health. In a two-year field experiment, we implemented five amendment treatments increasing in carbon: an untreated control, an unamended weed-free control, rye hay adding between 3,500 kg C·ha\(^{-1}\) and 3,300 kg C·ha\(^{-1}\) each year, sawdust adding between 4,300 kg C·ha\(^{-1}\) and 5,000 kg C·ha\(^{-1}\) each year, and a rye hay and sawdust combined treatment adding between 7,700 kg C·ha\(^{-1}\) and 8,500 kg C·ha\(^{-1}\) each year. We planted corn and soybean, and each treatment was replicated 5 and 6 times, respectively. In each season, we measured weed and crop biomass and weed species community composition. We measured soil respiration and nitrogen availability to monitor nitrogen immobilization. Soil health was measured with the Cornell Soil Health Test and microbial community composition was measured using amplicon sequencing. Amendments with a high carbon:nitrogen ratio (C:N) improved soybean crop competition over the weed community. The weed species composition in soils treated with low C:N amendments had higher leaf area index and nitrogen-responsiveness. Nitrogen availability was
lowest in plots treated with the highest C:N ratio amendment. Increasing carbon improved soil health metrics, but the microbial community composition was more affected by the rye hay treatment. Targeted nitrogen immobilization may improve leguminous crop competition over certain weed communities in an integrated weed management program.

4.2 Introduction

An important aspect of weed control is proper fertilization. In annual cropping systems, the most abundant weeds are nitrophilous, meaning that they grow well in high-nitrogen soils (Costea et al., 2004; Little et al., 2021; Moreau, 2014). Some of these species can outcompete crops for nitrogen and have higher nitrogen use efficiencies, which can lead to lower crop yields in fertilized soil relative to unfertilized soil at high weed densities (Di Tomaso 1995; Little et al. 2021). Once a weed accumulates more nutrients than a crop, it can become a stronger competitor for additional resources, such as light (Di Tomaso, 1995). Weeds can be especially competitive over crops with inorganic fertilizer use (Saberali and Mohammadi, 2015).

Weed scientists have experimented with varying the rates, forms, timing, and location of fertilizer applications to reduce nutrient uptake by weed species and enhance the competitive ability of crops over weeds. Studies that vary fertilization rates have produced mixed results depending on the nitrogen responsiveness of the crop and weed species (Blackshaw and Brandt, 2008). In some cases, reduced fertilization increased crop yield by reducing competition from weeds (Blackshaw et al., 2003; Davis and Liebman, 2001; Wortman et al., 2011). In other cases, there was no effect of nutrient availability on crop yield (Barker et al., 2006; Wortman et al., 2011). And in still others,
crop yield increased with increased fertilization rates, despite increased weed growth (Anderson et al., 1998; Juroszek et al., 2004). Little et al. (2021) recently developed a framework to help predict the effects of fertilization on weed competition and crop yield. According to this framework, key sources of variation in the effects of fertilization on crops and weeds include relative responsiveness to added nutrient, relative competitiveness, and relative shading ability under high fertility.

Another way to manage the growth of nitrophilous weeds is through nitrogen immobilization. Nitrogen immobilization is a microbially-mediated process (Schimel and Bennett, 2004). Carbon amendments added to the soil increase the growth of the soil microbial community (Zak et al., 1994). As the microbes grow, they also take up available soil nitrogen in order to maintain their stoichiometric ratio of carbon to nitrogen. This process leaves less nitrogen available to plant roots and may reduce weed growth.

Managing plant growth through nitrogen immobilization was first studied as a way to restore native plant growth in natural ecosystems invaded by non-native plants. In a recent review, the potential of using this strategy in agricultural landscapes was presented (Chapter 1 of this dissertation). Nitrogen immobilization may help explain the efficacy of several existing agricultural weed management strategies that utilize high carbon substrates. Many perennial cropping systems, such as strawberries (Fragaria × ananassa) (Pritts and Hadley, 1998), raspberries (Rubus idaeus L.) (Trinka and Pritts, 1992), blueberries (Vaccinium corymbosum L.), and apples [Malus domestica (Suckow) Borkh.] (Merwin et al., 1995), rely on high-carbon mulches to help manage weeds. Terminated cover crops can also immobilize nitrogen and nitrogen immobilization may
contribute to their weed-suppressive ability (Pittman et al., 2020; Wells et al., 2013; Williams et al., 2018). Mechanisms other than nitrogen immobilization, including physical impedance (Teasdale and Mohler, 2000; Creamer et al., 1996), light quantity and quality (Teasdale, 1993), changing microclimatic conditions (Teasdale et al., 2007), and allelopathy (Scavo and Mauromicale, 2021), certainly contribute to weed suppression exhibited by these tools. Nitrogen immobilization, however, likely plays an important role in all these strategies and is the focus of this study.

Carbon amendments, which can cause nitrogen immobilization, are also a critical component of soil biological functioning (Lal, 2014, 2016). Soil biological functioning is associated with diverse benefits such as improved aggregate stability, leading to increased water holding capacity, porosity, and water infiltration; increased nutrient cycling; improved cation exchange capacity; and increased crop yields (Thangarajan et al., 2013; Luo et al., 2018). Many bacterial and fungal endophytes, saprophytes, hyperparasites, and arbuscular mycorrhizal fungi can induce plant defense mechanisms helping protect plants from disease (Raaijmakers et al., 2008). Some microbes are microbial antagonists or microbial parasites, which help protect plants from pathogenic microbes. Many Trichoderma, Gliocladium, and non-pathogenic Fusarium (all Ascomycota) are antagonistic fungi (Harman et al., 2004; Raaijmakers et al., 2008). Many of the Pseudomonas (Proteobacteria), Burkholderia (Proteobacteria), Bacillus (Firmicutes), and Actinobacteria are antagonistic bacteria (Barka et al., 2015; Raaijmakers et al., 2008). Bacillus can also improve macronutrient cycling by solubilizing phosphorous-containing potassium compounds (Song et al.,
These are some of the ways that the soil biological community improves plant growth and soil health (Chaparro et al., 2012).

Soil health, as defined by the United States Department of Agriculture (USDA), is “the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans” (USDA, 2022). Soil health is a framework that considers the chemical, physical, biological, and ecological components of the soil (Lehmann et al., 2020) and is a key concept in creating sustainable cropping systems. It is probable that stimulating nitrogen immobilization with high carbon amendments will also improve soil health by improving soil biological functioning and changing the soil microbial community composition.

There were two main objectives of this research. The first objective was to test the efficacy of nitrogen immobilization as a tool for managing weeds in different field cropping systems. We hypothesized that amendments with higher carbon:nitrogen ration (C:N) added to the soil would increase nitrogen immobilization and reduce weed growth to a greater extent. This weed suppression would be greater for nitrophilous weeds, thus altering weed community composition. We predicted that soybeans \(\text{Glycine max (L.) Merr.}\) would be more competitive in nitrogen immobilized soils since soybeans can fix atmospheric nitrogen and are less reliant on soil nitrogen availability. We predicted that corn \(\text{Zea mays L.}\) growth would decrease as nitrogen immobilization increased since modern corn varieties are highly responsive to nitrogen (Liu et al., 2022) and have higher nitrogen recommended rates than many other crops (Mylavarapu et al., 2021). The second objective was to test whether high rates of carbon addition increase soil health ratings. We hypothesized that higher carbon inputs would stimulate
microbial growth and activity and alter microbial community composition. We predicted that these compositional changes would be correlated with improved soil health and function as more carbon was added to the soil.

4.3 Materials and methods

4.3.1 Field set-up

Field trials were initiated in the spring of 2020 at the Homer C. Thompson Vegetable Research Farm in Freeville, NY, USA (42°31’01.074” N, 76°20’03.282” W). The soil is classified as Howard gravelly loam (loamy-skeletal, mixed, active, mesic Glossic Hapludalf) (USDA, 2020). Baseline soil samples were submitted to the Cornell Nutrient Analysis Laboratory at the beginning of the study and nutrient levels were within recommended ranges for corn and soybean (Appendix Table 4.1). The field was previously planted to spinach (Spinacia oleracea L.) in 2019 and was winter seeded with rye (Secale cereale L.) in 2019 and 2020. In early May 2020 and 2021 rye was chopped at 15 to 20 cm height and removed from the field before plowing. Soybean and corn were planted separately, in plots arranged in a complete randomized block design. Within each block there were five treatments: sawdust amended, rye hay amended, rye hay and sawdust combined, nontreated control, and unamended weed-free control. Soil amendment rates and carbon and nitrogen characteristics are listed in Table 4.1. Amendments were spread starting mid-May, rototilled to about 15 cm deep, and then soybean and corn were planted on 27 May in 2020 and 24 May in 2021 in rows oriented north to south. Rainfall was below average in 2020, so supplemental irrigation was provided as needed. In 2021, rainfall was above average, so no supplemental irrigation was used (Appendix Fig. 4.1).
Table 4.1. Carbon (C) and nitrogen (N) content of amendments added to the soil in 2020 and 2021.

<table>
<thead>
<tr>
<th></th>
<th>Fresh biomass (g)</th>
<th>Dry biomass (g)</th>
<th>C (%)</th>
<th>N (%)</th>
<th>C:N</th>
<th>Application rate (kg C·ha(^{-1}))*</th>
<th>Application rate (kg N·ha(^{-1}))*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2020</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rye hay</td>
<td>40,848</td>
<td>9,386</td>
<td>45.5</td>
<td>2.4</td>
<td>19</td>
<td>3,558</td>
<td>187</td>
</tr>
<tr>
<td>Sawdust</td>
<td>38,608</td>
<td>13,200</td>
<td>45.7</td>
<td>0.2</td>
<td>288</td>
<td>5,027</td>
<td>17</td>
</tr>
<tr>
<td>Sawdust &amp; rye hay</td>
<td>79,456</td>
<td>22,586</td>
<td>45.6</td>
<td>1.1</td>
<td>41</td>
<td>8,585</td>
<td>209</td>
</tr>
<tr>
<td><strong>2021</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rye hay</td>
<td>41,935</td>
<td>18,034</td>
<td>42.8</td>
<td>2.1</td>
<td>21</td>
<td>3,349</td>
<td>159</td>
</tr>
<tr>
<td>Sawdust</td>
<td>42,434</td>
<td>11,219</td>
<td>39.6</td>
<td>0.2</td>
<td>221</td>
<td>4,352</td>
<td>20</td>
</tr>
<tr>
<td>Sawdust &amp; rye hay</td>
<td>84,369</td>
<td>29,253</td>
<td>41.6</td>
<td>1.4</td>
<td>30</td>
<td>7,701</td>
<td>257</td>
</tr>
</tbody>
</table>
*To convert to g·m\(^{-2}\) multiply by 0.1

Soybeans were planted at a 76 cm between-row and 4 cm within-row spacing. The soybean planting was divided into six blocks. Each block contained all five amendment treatments. Soybean plots were 6 m wide and 4 m long, which contained eight crop rows, and included a split-plot treatment in which half of each plot (four crop rows) was inoculated with rhizobia and half remained uninoculated. The soybean variety was 01072245 Roundup Ready 2 Xtend, RoundupReady2Yield Technology (AsGrow AG20x9, Bayer, Whippany, NJ), which was not pre-inoculated. Soybeans planted in inoculated plots were inoculated with rhizobia at a rate of 9.4 g·kg\(^{-1}\) seed (N-Dure Soybean, Verdesian, Cary, NC) about 10 minutes before planting. However, in 2020, at harvest, we dug three soybean plants from each plot, washed the roots, and counted root nodulation and there was no difference in nodulation rate by inoculation treatment (df=1, F-value=0.004, p-value=0.95). This indicates that there was an endemic population of *Bradyrhizobium japonicum* in the soil, which successfully colonized the non-inoculated soybeans, nullifying the inoculation treatment. Data were
therefore combined across inoculation treatments during some analyses, for a total of 12 replicates.

There were five blocks of silage corn and each block contained all five amendment treatments for a total of 25 plots. Each plot was 3 m wide and 4 m long to accommodate four crop rows. The corn was a hybrid blend of 95% FS 46R64VT2P and 5% VNS RR (VTDouble PRO Rib Complete, Monsanto, St. Louis, MO). Corn was planted at a 76 cm between-row and 20 cm within-row spacing. At planting 448.4 kg·ha\(^{-1}\) of N from 10-20-20 banded fertilizer (plant blended, Phelps Supply Inc., Phelps, NY) was applied 5 cm deep.

4.3.2 Weed communities

Seeds from four weed species collected from local farms were spread in each plot in early June 2020: Powell amaranth (*Amaranthus powellii* S. Watson), velvetleaf (*Abutilon theophrasti* Medik.), white clover (*Trifolium repens* L.) and ivyleaf morningglory (*Ipomoea hederacea* Jacq.). Powell amaranth and velvetleaf were expected to have reduced growth in carbon amended soils, but white clover and ivyleaf morningglory were not expected to show a response. Pre-weighed seeds from all four species were combined into a screw-top bottle (HDPE Plastic Wide Mouth, Thermo Scientific Nalgene, Rochester, NY), mixed with 120 mL of paver sand (Multi-purpose patio/paver sand, Pavestone, Atlanta, GA) to consistently distribute the different-sized seeds within the bottle, and then distributed evenly across each plot. All plots were raked to improve seed-soil contact.

Weed surveys were conducted in early August of each year of the experiment. A 0.25 m\(^2\) quadrat was placed 1 meter into the third crop row of each plot from the
southeast corner. The quadrats were 0.33 m wide and 0.75 m long and straddled the crop row, with 0.375 m extending into the inter-row space. In each quadrat, all weeds with at least one true leaf were identified to species and clipped at the soil surface, dried at 60°C to a constant weight, and then weighed. Only weeds that rooted within the quadrat were counted.

4.3.3 Field measurements

Soil nitrogen availability was measured using a method adapted from the Kellogg Biological Station Long-Term Ecological Research (LTER) program (http://lter.kbs.msu.edu/protocols/105). Anion and cation resin exchange membranes (Membranes International, Ringwood, NJ) were cut into strips 10 cm long and 2.5 cm wide. To charge the exchange strips, they were soaked in a 0.5 M HCl bath for one hour, stirring every 10 minutes. Then, strips were rinsed with ultrapure water and soaked in a 0.5 M NaHCO$_3$ bath for five hours, which was changed every hour. Strips were rinsed with ultrapure water a final time and stored at 4°C in sealed plastic bag to prevent drying. Anion and cation strips were charged and stored separately.

Strips were deployed in the field for periods of three weeks. In 2020 two measurements were taken: late June, and early August. In 2021 four measurements were taken: late June, mid July, early August, and late August. Strips were buried vertically 9.5 cm deep and approximately 15 cm on either side of the crops in the between row space. Care was taken to reduce soil disturbance and to ensure good strip-soil contact. After collection from the field, strips were rinsed with ultrapure water to remove visible soil particles and stored at 4°C until extraction. Any strips that had been eaten in the field were measured to determine the area of strip missing.
Soil respiration was measured using a soil gas flux survey chamber and CO$_2$ analyzer (Smart Chamber and LI-870 CO$_2$/H$_2$O Analyzer, LI-COR, Lincoln, NE). Soil collars were made from SDR35 PVC pipe, 20 cm in diameter and 10 cm tall. Collars were installed in the field in late July 2020 and early June 2021, leaving about 1 to 3 cm of the collar above the soil surface. The average offset height of each collar was integrated into the measurement of respiration per soil volume over 120 s. Respiration was measured twice in 2020 (late July and August) and four times in 2021 (mid-June, July, August, and early September). Measurements were taken on consecutive mornings for corn and soybean, at least 5 days after collar installation and at least 24 hours after removing any weeds from the collar.

4.3.4 Crop analyses

Corn and soybean samples were harvested in late September to early October 2020, and late September 2021. Samples were collected from the third crop row of each plot relative to the southeast corner, the row next to which weeds had been collected. Within a two-meter section in the middle of the row, all aboveground crop biomass was harvested, counted, and then dried at 60°C to a constant weight. Soybean pods were shelled by hand in 2020 and with a thresher in 2021 (LD 350, Wintersteiger, Innkreis, Austria) to measure seed yield. The entire corn stalk was weighed together. In 2020, the roots of three soybean plants within each harvested row were removed from the soil, carefully washed, and photographed. Root nodules were counted from the photographs.

4.3.5 Lab analyses

Before harvest, two soil samples were collected and homogenized from each corn and soybean plot. Samples were collected from the between row space of the two
middle rows, using a soil core sampler (AMS Inc., Americana Falls, ID) which was 2 cm wide to a depth of 13 cm. One sample from each plot was collected and discarded before keeping samples to “rinse” the core. Samples were stored on ice in the field and then transferred and stored at 3°C (Setpoint temperature control model SP-322; Goldline, N. Kingstown, RI) until processed.

Soil was sieved through a 2-mm sieve and further divided for future analyses. One aliquot was frozen at –20°C to be used for DNA extraction while another aliquot was set out to air dry. Sieves were rinsed with water, wiped with ethanol, and dried between samples. Gravimetric soil moisture was calculated by weighing a 10 g subsample of fresh soil into a tin cap and drying at 105°C in an oven (Isotemp Oven; Fisher Scientific, Suwanee, GA) for 24 hours before reweighing.

To measure pH, 3.0 ± 0.1 g of air-dried soil was mixed with 6 mL of deionized water in a 15 mL falcon tube and shaken on a reciprocal shaker (E6000 Medium-Duty; Eberbach Corporation, Ann Arbor, MI) at 180 revolutions per minute (rpm) for 60 minutes. Tubes were removed from the shaker and allowed to sit for at least 10 minutes before pH measurements were taken with a pH meter (Orion Star A215, Thermo Scientific, Waltham, MA).

Soil carbon and nitrogen were measured using air-dried and finely ground soil. The grinding apparatus was rinsed with ethanol between samples to prevent cross-contamination. About 30 mg of powdered soil was weighed into tin capsules and submitted to the Cornell Stable Isotope Lab for percent carbon (C), nitrogen (N), $^{13}$C, and $^{15}$N analysis on an isotope ratio mass spectrometer (Delta V, Thermo Scientific,
Both anion and cation resin strips were mixed with 70 mL of 1 M KCl in an acid-washed sample cup. Cups were shaken for 24 and 22 hours, in 2020 and 2021 respectively, 180 rpm on a reciprocal shaker (MaxQ 4000, Thermo Scientific, Waltham, MA) then decanted into 15 mL falcon tubes. Collected extract from all samples was stored frozen at –20°C until analysis. Nitrate and ammonium were quantified using the colorimetric method described by Hood-Nowotny et al. (2010). Samples were analyzed on a microplate reader (Synergy HT; Bio-Tek Instruments, Winooski, VT) and compared to a standard curve to calculate concentration.

In 2021, a subset of soil collected from the non-inoculated soybean and corn plots were submitted to the Cornell Soil Health Lab for a soil health assessment. Soil health indicator tests included: aggregate stability, organic matter, autoclaved-citrate extractable (ACE) soil protein index, active carbon, and macro- and micro- nutrients. Depth to compaction was also measured by recording the depth at which 21.1 kg·cm\(^{-2}\) of penetration resistance was reached using a penetrometer (AgraTronix Soil Compaction Tester, Streetsboro, OH, USA) (Duiker, 2002).

4.3.6 Microbial amplification

Bacterial and fungal community composition were assessed using high-throughput amplicon sequencing. DNA was extracted from approximately 200 mg of soil using the Qiagen DNeasy soil DNA extraction kit (Beverly, MA) following manufacturer instructions. The V3-V4 region of the 16S rRNA gene (16S) was targeted using 341F and 805R primers for bacterial identification (Herlemann et al., 2011). The
ITS2 region of the fungal internal transcribed spacer gene (ITS) region was targeted using the ITS1F and 58A2R primers for fungal identification (Gardes and Bruns, 1993; Martin and Rygiewicz, 2005). Amplified samples were cleaned, indexed, and pooled following the procedure detailed by Garcia et al. (2022). Pooled samples were sequenced on the Illumina MiSeq at the Cornell Genomics Facility (Ithaca, NY) using a 500-cycle MiSeq Reagent Kit v.2 for the ITS pool and a 600-cycle MiSeq Reagent Kit v.3 for the 16S pool.

Sequence merging and filtering were performed in Qiime2. Reads were demultiplexed, and paired ends were merged and trimmed of primers using q2-demux. Sequences were then denoised into amplicon sequence variants (ASVs) using DADA2 (Callahan et al. 2016). The ASVs were clustered using de-novo q2-vsearch, using 97% shared identity as the cutoff, to create a table of assigned operational taxonomic units (OTUs). The OTU table was used to create a feature table using q2-feature-table. Bacterial and fungal classifiers was trained with a Naïve Bayes classifier trained on the 99% Silva 138 database for bacteria (Bokulich et al., 2018; Robeson et al., 2020) and the UNITE 13.8 database for fungi (Abarenkov et al., 2010), and then taxonomy was assigned to sequences in the taxonomy table using classify-sklearn.

4.3.7 Statistical analyses

All statistical analyses were performed in R version 4.1.2 (R core team, 2021). Corn and soybean yields were analyzed separately using linear mixed-effects models (function lmer, in package lme4, version 1.1-31) with soil amendment treatment, year, weed biomass, and the interaction of weed biomass and amendment as fixed effects. The model for soybean yield also included an inoculation treatment and the interaction
of inoculation and amendment. Block was always included as a random effect. The
correlation between soybean seed yield and soybean aboveground biomass was
calculated using the Spearman method. The same fixed and random effects were
included in models of total weed biomass across all plots. The crop biomass per meter
of crop row and the weed biomass per square meter were summed and then percent crop
biomass of total biomass was calculated. The crop biomass, weed biomass, and percent
crop biomass of total plant growth were analyzed separately for each year, using a linear
mixed-effects model which included all the aforementioned covariates except year.

An indicator species analysis (function multipatt, in package indicspecies,
version 1.7.12) was based on weed biomass per species per plot, excluding weed-free
control plots. To assess weed species traits, representative Ellenberg N index values,
seed masses, and specific leaf areas were researched for each weed species in our
dataset. Data were preferentially taken from Bàrberi et al. (2018), then from the TRY
Plant Trait Database (Kattge et al., 2020), then from the chart in Manage Weeds on Your
Farm: A Guide to Ecological Strategies (Mohler et al. 2021). In Manage Weeds on Your
Farm, the Ellenberg N index was not reported but responsiveness to nutrients was
categorized as low, moderate, or high. Ellenberg N indexes of 2, 5, and 8 were assigned
to those categories, respectively. Specific leaf area of large crabgrass [Digitaria
sanguinalis (L.) Scop.] was taken from Garnier et al. (1997). Representative trait values
for each species were multiplied by the number of that species found in each plot. The
resulting values were summed across all weed species, then divided by the total number
of weeds found in the plot to calculate the weighted mean trait value for the weed
community. Data from corn and soybean plots were pooled and analyzed together.
Weighted mean trait values were used as dependent variables for linear mixed-effects models as described above.

Soil respiration, nitrate, and ammonium were analyzed for each crop separately. For corn treatments, the amendment was the only fixed effect. For soybean treatments, the fixed effects were amendment, inoculation, and the interaction between amendment and inoculation. Post-hoc analyses were done using Tukey’s HSD (function cld, in package lsmeans, version 2.27-62). Individual soil health indicator test values from the Cornell Soil Health Test were rated on a scale of 0 to 100 based on their desirability (Moebius-Clune et al., 2016). These ratings were averaged across all indicator tests to create the overall soil health indicator score. This overall soil health score and the raw values (not rated on the 0 to 100 scale) were analyzed using linear mixed-effects models as described above. Raw values were also centered and scaled and a principal components analysis was run (function prcomp, in package stats, version 3.6.2). The first two eigenvectors were plotted to show the dominant relationship between the plots. The rotation data were plotted to show the influence of each soil health indicator test on the overall soil health score.

To prepare data for microbial community analyses, samples with less than 1,000 reads were removed. Data were randomly subsampled to give an equal number of reads per sample (function rrarefy, in package vegan, version 2.6-4) and feature counts were converted to percentages. Percent abundance was converted to a Bray-Curtis distance matrix (function vegdist, in package vegan, version 2.5-7). A principal coordinates analysis (PCoA) was performed on the distance matrix (function cmdscale, in package stats, version 4.1.2). A Permutational Multivariate Analysis of Variance
(PERMANOVA) using 999 permutations was run to determine significant differences between treatments with pseudo P-values (function `adonis2`, in package vegan, version 2.5-7).

Differential abundances of OTUs based on rye or no-rye amendment were calculated in two ways. The first workflow used a Dirichlet-multinomial model with transformed (centered log-ratio transformation) non-rarefied abundance data (function `aldex`, in package ALDEx2, version 1.4.0) (Fernandes et al., 2014). The second workflow detected multivariable associations using linear models of normalized (total-sum scaling) and log transformed non-rarefied abundance data with a Benjamini-Hochberg correction method to reduce the false discovery rate (function `Maaslin2`, in package Maaslin2, version 1.8.0) (Mallick et al., 2021). Additionally, a Random Forest machine learning algorithm was used (function `randomForest`, in package randomForest, version 4.6-14) to create a predictive model of rye-amended vs. no-rye soils. Random Forest was run on centered log-ratio values of OTUs with greater than 2% abundance across all the plots.

4.4 Results

4.4.1 Crop and weed aboveground biomass

Total soybean biomass was highly correlated to soybean seed yield (R=0.98). Soybean biomass was significantly affected by amendment treatment (df=4, F-value=6.97, p<0.0001) and year (df=1, F-value=6.85, p=0.01); however, it was not affected by inoculation treatment, total weed biomass, or interactions between those variables and treatments (Table 4.2). Inoculation treatment did not affect nodulation rates in 2020 (df=1, F-value=0.004, p-value=0.95), however nodulation rates were
affected by the soil amendment treatment (df=4, F-value=2.87, p-value=0.03). The weed-free control had a lower nodulation rate than the sawdust and rye hay treatment and all other treatments had an intermediate nodulation rate. Within year, all amendment treatments reduced soybean biomass compared to the weed-free control except sawdust and rye hay combined in 2020 (df=4, F-value=10.06, p<0.0001) (Fig. 4.1). In 2020, the nontreated control soils had the lowest soybean biomass but in 2021, rye hay-amended soils had the lowest soybean biomass. Total weed biomass in soybean plots also varied based on amendment treatment (df=4, F-value=146.6, p<0.0001), but did not vary with year or inoculation treatment and their interaction (Table 4.2). Within year, all soil amendment treatments had higher weed biomass than the weed-free control except sawdust amended plots both years and sawdust and rye hay combined plots in 2020. Rye hay amended plots had the highest weed biomass in both years.

Total corn biomass was not significantly affected by any independent variable; however, it was marginally affected by amendment (df=4, F-value=2.21, p=0.09) and year (df=1, F-value=3.68, p=0.06) (Table 4.2). In 2020 sawdust and rye hay combined plots reduced corn biomass relative to the weed-free control (df=4, F-value=5.28, p=0.007) but in 2021 there was only a marginal effect of soil amendment treatment on yield (df=4, F-value=2.87, p=0.06) (Fig. 4.2). Total weed biomass in corn plots varied based on amendment treatment (df=4, F-value=68.3, p<0.0001), but did not vary with year. In 2020, all amendment treatments had increased weed biomass compared to the weed-free control but in 2021 only the rye hay and sawdust and rye hay combined plots had higher weed biomass than the weed-free control.
Table 4.2. P-values, degrees of freedom (df), and F-values of crop and weed aboveground biomass from a type II ANOVA of a linear model for each independent variable included in the model.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>df</th>
<th>F-value</th>
<th>P-value</th>
<th>df</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soybean</td>
<td></td>
<td></td>
<td>Corn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amendment</td>
<td>4</td>
<td>6.9741</td>
<td>&lt;.0001</td>
<td>4</td>
<td>2.2116</td>
<td>0.08691</td>
</tr>
<tr>
<td>Inoculation</td>
<td>1</td>
<td>2.3586</td>
<td>0.12766</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Weed biomass</td>
<td>1</td>
<td>1.2323</td>
<td>0.26955</td>
<td>1</td>
<td>0.8832</td>
<td>0.35341</td>
</tr>
<tr>
<td>Year</td>
<td>1</td>
<td>6.8523</td>
<td>0.01019</td>
<td>1</td>
<td>3.6753</td>
<td>0.06316</td>
</tr>
<tr>
<td>Amendment × inoculation</td>
<td>4</td>
<td>0.6193</td>
<td>0.64978</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Amendment × weed biomass</td>
<td>4</td>
<td>1.9027</td>
<td>0.1156</td>
<td>4</td>
<td>0.6902</td>
<td>0.6034</td>
</tr>
<tr>
<td>Soybean weeds</td>
<td>4</td>
<td>146.61097</td>
<td>&lt;.0001</td>
<td>4</td>
<td>68.27369</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Inoculation</td>
<td>1</td>
<td>1.09508</td>
<td>0.2978</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Year</td>
<td>1</td>
<td>0.06571</td>
<td>0.7982</td>
<td>1</td>
<td>0.40776</td>
<td>0.5267</td>
</tr>
<tr>
<td>Amendment × inoculation</td>
<td>4</td>
<td>0.48764</td>
<td>0.7448</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Percent soybean biomass of total plant growth was affected by soil amendment treatment in both 2020 (df=4, F-value=25.96, p<0.0001) and 2021 (df=4, F-value=47.89, p<0.0001) (Fig. 4.3). Percent corn biomass of total plant growth was not affected by soil amendments in 2020 (df=4, F-value=2.88, p=0.06) but was in 2021 (df=4, F-value=8.36, p=0.0008) (Fig. 4.3). Weed-free control plots all had the highest percentage of crop growth, followed by sawdust amended plots. Rye hay amended plots consistently had the lowest percentage of crop growth.
Figure 4.1. Soybean crop yield and total weed biomass in soils with different amendments. Bars labeled with different letters indicate significantly different means (p<0.05) by treatment.

Figure 4.2. Corn crop yield and total weed biomass in soils with different amendments. Bars labeled with different letters indicate significantly different means (p<0.05) by treatment.
Figure 4.3. Percent soybean and corn crop yield relative to the total aboveground plant biomass within a square meter in soils with different amendments. Bars labeled with different letters indicate significantly different means (p<0.05) by treatment.

4.4.2 Weed community composition

Using an indicator species analysis, species indicative of soil amendment treatments (pooled across corn and soybean plots) were identified for both 2020 and 2021 (Table 4.3). All the species identified were indicative of rye hay-amended plots except field pennycress (*Thlaspi arvense* L.), which was indicative of sawdust and rye hay combined plots in 2021. Only *Amaranthus* spp. and common lambsquarters (*Chenopodium album* L.) in 2021 were solely indicative of rye hay amended plots; all other species were indicative of multiple amendment treatments. In 2020, common purslane (*Portulaca oleracea* L.) was indicative of the nontreated control plots, rye hay
plots, and sawdust and rye hay combined plots. In 2021, shepherd’s purse \( \textit{Capsella bursa-pastoris} \) (L.) Medik.) and witchgrass \( \textit{Panicum capillare} \) L.) were indicative of these treatments. \( C. \textit{bursa-pastoris} \) was only indicative of nontreated control and rye hay plots in 2020. Chickweed \( \textit{Stellaria media} \) (L.) Vill.] and dandelion \( \textit{Taraxacum officinale} \) Weber ex Wiggins) were both indicative of rye hay plots and sawdust and rye hay combined plots in 2021. No species were identified as indicative of sawdust-amended plots.

In 2021, weighted average weed species traits were also affected by soil amendment treatment. Seed weight was not affected by any treatment variable (data not shown). However, the Ellenberg N index (df=3, F-value=6.99, p=0.0005) and specific leaf area (df=3, F-value=5.37, p=0.003) were both affected by soil amendment treatment (Fig. 4.4). Both showed a similar trend with the highest value in rye hay-amended plots and the lowest value in sawdust-amended plots, although no treatments were significantly different according to post-hoc analysis of specific leaf area.

Table 4.3. Significant indicator species identified in plots with different amendment treatments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Amendment</th>
<th>Stat</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2020</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Portulaca oleracea}</td>
<td>Weedy control</td>
<td>0.955</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Rye hay</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sawdust and rye hay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Capsella bursa-pastoris}</td>
<td>Weedy control</td>
<td>0.888</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Rye hay</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2021</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Capsella bursa-pastoris}</td>
<td>Weedy control</td>
<td>0.934</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Rye hay</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sawdust and rye hay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Panicum capillare}</td>
<td>Weedy control</td>
<td>0.832</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Rye hay</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sawdust and rye hay</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Stellaria media**
- Rye hay: 0.727, 0.005
- Sawdust and rye hay

**Taraxacum officinale**
- Rye hay: 0.711, 0.015
- Sawdust and rye hay

**Thlaspi arvense**
- Sawdust and rye hay: 0.467, 0.04

**Amaranthus spp.**
- Rye hay: 0.842, 0.01

**Chenopodium album**
- Rye hay: 0.732, 0.005

Figure 4.4. Average weed species traits in plots treated with different amendments. Trait values were retrieved from the literature for each species and weighted by the number of that species found in a plot. Boxplots labeled with different letters indicate significantly different means (p<0.05) by treatment.

4.4.3 **Indicators of nitrogen immobilization**
Indicators of nitrogen immobilization were measured both by soil CO$_2$ emissions as well as soil nitrate and ammonium. Soil CO$_2$ is a measure of microbial respiration, which is an indicator of soil microbial activity. Soil respiration was significantly responsive to soil amendment treatments at every sample date except June (soybean soils) and September (corn soils) in 2021 (Table 4.4). Total respiration measured in each year was also significantly different based on amendment treatment for both corn (df=5, F-value=10.4, p=0.0002 in 2020 and df=5, F-value=7.6, p=0.0007 in 2021) and soybean (df=6, F-value=13.9, p>0.0001 in 2020 and df=6, F-value=8.4, p>0.0001 in 2021). Respiration was consistently highest for the soil amended with sawdust and rye hay combined (Fig. 4.5), although it was not significantly different from rye hay-amended soil for corn in 2021, sawdust-amended soil for soybean in 2020, or rye hay- or sawdust-amended soil for soybean in 2021.

Increased microbial respiration did not always correspond with decreased nitrogen availability. Total nitrate measured over each year was significantly different based on amendment treatment for both corn (df=5, F-value=3.7, p=0.03 in 2020 and df=5, F-value=4.9, p=0.009 in 2021) and soybean (df=6, F-value=3.5, p>0.01 in 2020 and df=6, F-value=6.2, p=0.0004 in 2021). Consistently, total nitrate was lowest in sawdust-amended soil and highest in rye hay-amended soil or unamended weed-free control soil (Fig. 4.6). Total ammonium availability was more variable. Total ammonium was significantly affected by amendment treatment in corn in 2021 (df=5, F-value=5.5, p=0.005) and soybean in 2020 (df=6, F-value=4.6, p=0.003). It was numerically lowest in sawdust-amended soil in 2020 but numerically lowest in the nontreated control for corn and in the unamended weed-free control for soybean in 2021.
Rye hay-amended soil had the highest ammonium availability for corn in 2021 and soybean in 2020 and 2021 (Fig. 4.7).

Table 4.4. Mean soil measurements (respiration, nitrate, and ammonium) in soils with different amendment treatments. Different letters indicate significantly different means (p<0.05) by treatment for that sample date.

<table>
<thead>
<tr>
<th></th>
<th>Weed-free control</th>
<th>Nontreated control</th>
<th>Rye hay</th>
<th>Sawdust and rye hay</th>
<th>Sawdust</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiration (ppm·s⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean 2020 July</td>
<td>2.75 (a)</td>
<td>3.49 (a)</td>
<td>4.27 (a)</td>
<td>7.16 (b)</td>
<td>5.35 (ab)</td>
</tr>
<tr>
<td>August</td>
<td>2.62 (a)</td>
<td>3.32 (ab)</td>
<td>3.77 (ab)</td>
<td>5.62 (c)</td>
<td>4.99 (bc)</td>
</tr>
<tr>
<td>June 2021</td>
<td>1.71</td>
<td>2.67</td>
<td>2.46</td>
<td>3.55</td>
<td>2.90</td>
</tr>
<tr>
<td>July</td>
<td>3.40 (a)</td>
<td>3.51 (a)</td>
<td>9.14 (a)</td>
<td>8.57 (a)</td>
<td>5.14 (a)</td>
</tr>
<tr>
<td>August</td>
<td>6.06 (a)</td>
<td>8.10 (a)</td>
<td>10.51 (a)</td>
<td>12.59 (a)</td>
<td>8.86 (a)</td>
</tr>
<tr>
<td>September</td>
<td>3.26 (abc)</td>
<td>2.98 (ab)</td>
<td>5.39 (bc)</td>
<td>7.51 (c)</td>
<td>2.79 (a)</td>
</tr>
<tr>
<td>Soybean 2020 July</td>
<td>2.18 (a)</td>
<td>2.15 (a)</td>
<td>2.40 (a)</td>
<td>4.70 (b)</td>
<td>2.61 (ab)</td>
</tr>
<tr>
<td>August</td>
<td>1.56 (a)</td>
<td>1.41 (a)</td>
<td>1.77 (ab)</td>
<td>2.25 (ab)</td>
<td>2.61 (b)</td>
</tr>
<tr>
<td>Corn 2021 June</td>
<td>1.48 (a)</td>
<td>1.47 (a)</td>
<td>2.72 (bc)</td>
<td>3.84 (c)</td>
<td>2.92 (ab)</td>
</tr>
<tr>
<td>July</td>
<td>1.81 (a)</td>
<td>3.24 (a)</td>
<td>16.24 (ab)</td>
<td>27.34 (b)</td>
<td>4.46 (a)</td>
</tr>
<tr>
<td>August</td>
<td>5.72 (a)</td>
<td>6.33 (a)</td>
<td>12.31 (ab)</td>
<td>16.94 (b)</td>
<td>5.97 (a)</td>
</tr>
<tr>
<td>September</td>
<td>4.98</td>
<td>3.48</td>
<td>6.01</td>
<td>9.89</td>
<td>4.44</td>
</tr>
<tr>
<td><strong>Nitrate NO₃⁻-N (ppm·w⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean 2020 July</td>
<td>0.0569 (a)</td>
<td>0.0549 (a)</td>
<td>0.1546 (b)</td>
<td>0.0774 (a)</td>
<td>0.0184 (a)</td>
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<td>August</td>
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<td>0.037</td>
<td>0.0788</td>
<td>0.0975</td>
<td>0.0262</td>
</tr>
<tr>
<td>June 2021</td>
<td>0.07539</td>
<td>0.03967</td>
<td>0.05929</td>
<td>0.05558</td>
<td>0.00671</td>
</tr>
<tr>
<td>July</td>
<td>0.01051 (b)</td>
<td>0.0039 (a)</td>
<td>0.00385 (a)</td>
<td>0.00268 (a)</td>
<td>0.00117 (a)</td>
</tr>
<tr>
<td>August</td>
<td>0.01662 (b)</td>
<td>0.00826 (ab)</td>
<td>0.01283 (ab)</td>
<td>0.00713 (a)</td>
<td>0.00621 (a)</td>
</tr>
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<td>September</td>
<td>0.0714 (b)</td>
<td>0.0331 (a)</td>
<td>0.0374 (ab)</td>
<td>0.0128 (a)</td>
<td>0.022 (a)</td>
</tr>
<tr>
<td>Corn 2020 July</td>
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<td>0.1713</td>
<td>0.3992</td>
<td>0.202</td>
<td>0.0572</td>
</tr>
<tr>
<td>August</td>
<td>0.0577 (bc)</td>
<td>0.0285 (ab)</td>
<td>0.0657 (a)</td>
<td>0.049 (abc)</td>
<td>0.0139 (a)</td>
</tr>
<tr>
<td>June 2021</td>
<td>0.09111 (b)</td>
<td>0.04636 (ab)</td>
<td>0.08163 (ab)</td>
<td>0.01625 (ab)</td>
<td>0.00689 (a)</td>
</tr>
<tr>
<td>July</td>
<td>0.00931 (b)</td>
<td>0.00372 (ab)</td>
<td>0.0063 (ab)</td>
<td>0.00483 (ab)</td>
<td>0.00176 (a)</td>
</tr>
<tr>
<td>August</td>
<td>0.00991</td>
<td>0.0032</td>
<td>0.0078</td>
<td>0.0066</td>
<td>0.00385</td>
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<tr>
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<td>0.01232</td>
<td>0.00779</td>
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<td>0.01056</td>
<td>0.00359</td>
</tr>
<tr>
<td><strong>Ammonium NH₄⁺-N (ppm·w⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean 2020 July</td>
<td>0.000134 (a)</td>
<td>0.000269 (a)</td>
<td>0.000729 (b)</td>
<td>0.000384 (a)</td>
<td>0.000155 (a)</td>
</tr>
<tr>
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<td>0.000557</td>
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<td>0.000164</td>
</tr>
<tr>
<td>June 2021</td>
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<td>0.000512</td>
<td>0.000492</td>
<td>0.000726</td>
<td>0.000481</td>
</tr>
<tr>
<td>July</td>
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<td>0.000165</td>
<td>0.000803</td>
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<td>0.000407</td>
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<tr>
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<td>0.000585</td>
<td>0.001063</td>
<td>0.000754</td>
<td>0.001163</td>
</tr>
<tr>
<td>September</td>
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<td>0.00319</td>
<td>0.0031</td>
<td>0.00278</td>
<td>0.00115</td>
</tr>
<tr>
<td>Year</td>
<td>Month</td>
<td>2020</td>
<td>2021</td>
<td></td>
<td></td>
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<td>------</td>
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<td></td>
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<td></td>
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<td>Mean</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO\textsubscript{2} (ppm s\textsuperscript{-1})</td>
<td>CO\textsubscript{2} (ppm s\textsuperscript{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2020</td>
<td>July</td>
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<td>0.000826</td>
<td></td>
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<td>August</td>
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<td>0.001021</td>
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<td></td>
</tr>
<tr>
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<td>June</td>
<td>0.00004 (a)</td>
<td>0.000351 (a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>0.000166</td>
<td>0.000377</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>August</td>
<td>0.000959</td>
<td>0.000736</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>0.000727</td>
<td>0.000652</td>
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</tbody>
</table>

Figure 4.5. Mean total soil respiration as affected by amendment treatment in 2020 and 2021. Measurements were taken twice in 2020 (July and August) and four times in 2021 (June, July, August, and September). Bars labeled with different letters indicate significantly different means (p<0.05) by treatment within year.
Figure 4.6. Mean total nitrate as affected by amendment treatment in 2020 and 2021. Measurements were taken twice in 2020 (July and August) and four times in 2021 (June, July, August, and September). Bars labeled with different letters indicate significantly different means (p<0.05) by treatment within year.
Figure 4.7. Mean total ammonium as affected by amendment treatment in 2020 and 2021. Measurements were taken twice in 2020 (July and August) and four times in 2021 (June, July, August, and September). Bars labeled with different letters indicate significantly different means (p<0.05) by treatment within year.

4.4.4 Soil health indices

Average soil health indicator scores were also affected by soil amendment treatment (df=3, F-value=5.37, p=0.003). Sawdust and rye hay combined plots had the highest soil health scores and unamended control plots had the lowest scores (Fig. 4.8A). Most soil health indicator tests were significantly affected by soil amendment treatment
(Table 4.5), except depth to hardpan, pH, aluminum, iron, and sulfur. Principal component analysis of centered and scaled values of soil health indicator tests demonstrates that the higher-rated sawdust and rye hay combined plots cluster together and are associated with higher organic matter, active carbon, ACE soil protein, and soil respiration as well as higher macro- and micro- nutrients such as magnesium, potassium, and percent carbon (Fig. 4.8B).

Table 4.5. The p-value and mean soil health indicator value by soil amendment treatment. Values labeled with different letters indicate significantly different means from other values within the row based on Tukey’s post-hoc analysis.

<table>
<thead>
<tr>
<th>Soil Health Indicator</th>
<th>p-value</th>
<th>Weed-free control</th>
<th>Untreated control</th>
<th>Rye hay</th>
<th>Sawdust and rye hay</th>
<th>Sawdust</th>
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<td>Organic matter (%)</td>
<td>&lt;0.0001</td>
<td>2.14 (a)</td>
<td>2.15 (a)</td>
<td>2.34 (a)</td>
<td>2.71 (b)</td>
<td>2.7 (b)</td>
</tr>
<tr>
<td>Soil respiration (ppm·s⁻¹)</td>
<td>&lt;0.0001</td>
<td>15.1 (a)</td>
<td>16.6 (a)</td>
<td>34.5 (bc)</td>
<td>46.3 (c)</td>
<td>18.2 (ab)</td>
</tr>
<tr>
<td>Active carbon (mg·kg soil⁻¹)</td>
<td>&lt;0.0001</td>
<td>360 (a)</td>
<td>347 (a)</td>
<td>417 (b)</td>
<td>466 (b)</td>
<td>446 (b)</td>
</tr>
<tr>
<td>Carbon (%)</td>
<td>0.0005</td>
<td>1.08 (a)</td>
<td>1.17 (ab)</td>
<td>1.25 (ab)</td>
<td>1.51 (b)</td>
<td>1.53 (ab)</td>
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<tr>
<td>Carbon:nitrogen (ratio)</td>
<td>0.0002</td>
<td>9.25 (a)</td>
<td>9.8 (ab)</td>
<td>9.39 (ab)</td>
<td>11.47 (b)</td>
<td>12.12 (ab)</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>&lt;0.0001</td>
<td>0.116 (a)</td>
<td>0.119 (ab)</td>
<td>0.133 (b)</td>
<td>0.132 (ab)</td>
<td>0.126 (b)</td>
</tr>
<tr>
<td>NO₃⁻ (ppm·w⁻¹)</td>
<td>&lt;0.0001</td>
<td>0.15 (c)</td>
<td>0.0738 (abc)</td>
<td>0.1006 (bc)</td>
<td>0.0388 (ab)</td>
<td>0.0319 (a)</td>
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<tr>
<td>Ace soil protein index (mg·g soil⁻¹)</td>
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<td>3.62 (b)</td>
<td>3.61 (ab)</td>
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<td>4.43 (c)</td>
<td>4 (bc)</td>
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<tr>
<td>Soil moisture (%)</td>
<td>&lt;0.0001</td>
<td>15.6 (a)</td>
<td>16 (ab)</td>
<td>16.5 (ab)</td>
<td>18.1 (c)</td>
<td>17.7 (bc)</td>
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<td>Phosphorous (mg·kg soil⁻¹)</td>
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<td>13.1 (a)</td>
<td>12.6 (a)</td>
<td>14.4 (a)</td>
<td>16.2 (a)</td>
<td>15.2 (a)</td>
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<tr>
<td>Potassium (mg·kg soil⁻¹)</td>
<td>&lt;0.0001</td>
<td>104 (a)</td>
<td>116 (ab)</td>
<td>204 (c)</td>
<td>248 (d)</td>
<td>142 (b)</td>
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<tr>
<td>Calcium (mg·kg soil⁻¹)</td>
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<td>924 (a)</td>
<td>886 (a)</td>
<td>950 (a)</td>
<td>982 (a)</td>
<td>1013 (a)</td>
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<tr>
<td>Copper (mg·kg soil⁻¹)</td>
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<td>0.119 (b)</td>
<td>0.119 (ab)</td>
<td>0.126 (ab)</td>
<td>0.125 (ab)</td>
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<td>Magnesium (mg·kg soil⁻¹)</td>
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<td>Manganese (mg·kg soil⁻¹)</td>
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<td>Zinc (mg·kg soil⁻¹)</td>
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<td>0.115 (a)</td>
<td>0.107 (a)</td>
<td>0.15 (a)</td>
<td>0.15 (a)</td>
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</table>
Figure 4.8. A.) Average soil health ratings for each plot treated with different amendments. Boxplots labeled with different letters indicate significantly different means (p<0.05) by treatment. B.) Principal component analysis (PCA) of centered and scaled soil health indicator values. Shape indicates crop, size indicates soil health rating, and color indicates amendment treatment. All indicators were significantly affected by treatment (p<0.05) except indicators outlined in red.
4.4.5 Microbial community composition

In both corn and soybean plots, microbial beta diversity clustered by treatment in a principal coordinates analysis (Fig. 4.9). The PERMANOVA results indicate significant associations between the microbial community and year (p-value=0.001 for bacteria and p-value=0.001 for fungi), soil amendment treatment (p-value=0.001 for bacteria and p-value=0.001 for fungi), and crop (p-value=0.005 for bacteria and p-value=0.001 for fungi). When beta diversity is analyzed separately for each year, or for only the soils where soil health was measured, soil amendment treatment and crop remain significant (Table 4.6).

Table 4.6. The p-values and degrees of freedom (df) for bacterial (16S rRNA region) and fungal (ITS2 region) beta diversity, measured with a PERMANOVA on a principal coordinates analysis of a Bray-Curtis distance matrix of OTUs.

<table>
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<tr>
<th>Dependent variable</th>
<th>Bacteria df</th>
<th>Bacteria P-value</th>
<th>Fungi df</th>
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<tr>
<td>Amendment × crop</td>
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<td></td>
<td>2021</td>
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<tr>
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<td>Amendment × crop</td>
<td>4</td>
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PERMANOVA revealed significant effects of soil amendment (pseudo p-value = 0.001 for bacteria and fungi), crop (pseudo p-value = 0.005 for bacteria and pseudo p-value = 0.001 for fungi), and year (pseudo p-value = 0.001 for bacteria and fungi) on microbial community composition.
The effect of soil amendment treatment appears to be largely driven by the rye hay amendment. The random forest algorithm accurately divides plots into rye hay-amended or no-rye hay plots using both bacterial and fungal communities. The Out Of Box (OOB) error rates for bacterial communities were 5.41% in 2020 and 3.33% in 2021. The OOB error rates for fungal communities were 8.24% in 2020 and 12% in 2021. These error rates are much smaller than the error rates using all five amendment treatments as categories (55.41% in 2020 and 51.67% in 2021 for bacteria and 24.71% in 2020 and 24% in 2021 for fungi). Using Maaslin2, OTUs with significantly different abundance between rye-amended and no-rye treatments were identified for bacteria and fungi in both years. Percent abundances in rye and no-rye plots are shown in Fig. 4.10 for the 10 OTUs with the lowest q-values and taxonomic information for these 10 OTUs is presented in Table 4.7. Despite the low random forest model error rates and the identification of OTUs with differential abundance between rye-amended and no-rye plots according to Maaslin2, the ALDEx2 approach did not identify any bacterial or fungal OTUs as differentially abundant.
Figure 4.10. The 10 bacterial (A) and fungal (B) OTUs whose abundances differed most significantly between rye-amended and no-rye plots. These OTUs were identified with multivariable associations with linear models (Maaslin2). Maaslin2 was run on non-rarefied abundance data, but percentage relative abundance data are shown. Identified taxa are ordered from most (top) to least (bottom) significantly different. Full taxonomic classification for each identified taxon can be found in Table 4.7.
Table 4.7. Top 10 bacterial and fungal operational taxonomic units (OTUs) with significantly different abundance between plots amended with rye hay and no-rye plots, as identified by the Microbiome Multivariable Association with Linear Models 2.0 algorithm. The 6-digit OTU identifiers correspond to the same 6-digit OTU identifiers in Figure 4.10.

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<th>Bacteria</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
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4.5 Discussion

4.5.1 C:N ratios changed crop-weed competition

Soil amendment C:N shifted crop-weed competition by altering the plant traits that conveyed competitive advantage in different soil environments. Low C:N amendments quickly selected for weed communities with functional traits that improved aboveground resource capture and nitrogen responsiveness. High C:N amendments conferred competitive advantage to crops with greater belowground investment before the weed community composition had time to shift.

In soils amended with high C:N amendments, we observed increased competitive advantage of soybeans over weeds. Soybean biomass constituted an increasing percentage of the total plant community growth as the C:N of amendments increased. Sawdust-amended plots had the highest percent crop biomass and rye hay-amended plots had the lowest in both years. This effect was driven both by higher soybean biomass in soils amended with higher C:N amendments, as well as reductions in total weed growth. These crop competition results from our study are consistent with other studies looking at soybean yield when grown in cover crop systems with low available nitrogen soils (Pittman et al., 2020; Wells et al., 2013; Williams et al., 2018). Soybeans are able to fix up to 300 kg N·ha$^{-1}$ or 95% of their own nitrogen from the atmosphere by forming a symbiotic relationship with rhizobia in the soil (Keyser and Li, 1992; Mourtzinis et al., 2018). This trait can convey a competitive advantage to soybeans over weeds, especially in low resource soils (van Heemst, 1985). Many factors affect biological nitrogen fixation rates, but it is well established that biological fixation increases as soil available nitrogen rates decrease (Keyser and Li, 1992; Siczek and
Lipiec, 2011). In 2020 we had the lowest nodulation rates in our weed-free control plots and the highest nodulation rate in the sawdust and rye hay plots. These rates seem more correlated with soil respiration and percent soil carbon – which were also highest for the sawdust and rye hay combined plots and lowest in the weed-free control plots – than nitrogen availability in our study. It seems likely that both percent soil carbon and soil nitrogen availability could play a role in soybean nodulation rates.

Corn, however, did not have an increase in competitive advantage with higher C:N amendments. The percent corn biomass of the total plant community growth had a similar trend to soybeans: with higher C:N amendments there was an increase in the percentage of corn biomass. However, this trend was driven by the changing competitive ability of the weed community, not the corn. Similar to soybean plots, total weed growth decreased in soils with higher C:N amendments, but unlike soybeans, corn biomass did not vary based on the C:N of the amendment.

Regardless of the crop planted, the weed community shifted consistently with soil amendments, and soils amended with low C:N amendments always had the highest percentage of weed growth. The weeds in these soils had a greater nitrogen responsiveness and higher leaf area index on average, which is consistent with the theory that nitrogen-responsive plants have more $r$-selected growth habits and faster population increases (Grime, 1977; Grime and Hunt, 1975; Moreau et al., 2014). It may follow that only ruderal species had time to respond to the amendment treatments within the two-year timeframe of this study, but that within a longer timeframe, high C:N amendments would have selected for slower-growing plants with greater belowground investment. Results from our indicator species analysis are consistent with this theory.
Most indicator species were associated with rye hay-amended plots and no species were indicative of sawdust, our highest C:N amendment. The literature review in Chapter 1 of this dissertation similarly found that native species growth was not faster in nitrogen-immobilized soils than in unamended soils, but invasive species growth was reduced while native species growth was unaffected by high C:N amendments.

These findings highlight the potential to utilize reverse fertilization in agriculture. In a highly managed field, nitrogen responsive weeds will become the most problematic. Reverse fertilization temporarily limits nitrogen availability, reducing the competitive advantage of these weeds. Before the weed community has time to naturally shift towards species with greater belowground investment, leguminous crops will have a competitive advantage.

4.5.2 High C:N ratio amendments immobilized nitrogen

We found evidence that increased microbial community activity can reduce plant available nitrogen depending on the C:N of the amendment. Both nitrate and ammonium availability were lowest in sawdust-amended plots (Fig. 6 and 7), indicating that the greatest nitrogen immobilization occurred with the highest C:N amendment. Sawdust and rye hay combined plots, which had the greatest total amount of carbon added to the soil, had greater microbial activity as indicated by the highest soil respiration rates (Fig. 5) but the highest respiration did not correspond to the lowest nitrogen availability. This implies that to stimulate the microbial community activity, carbon additions are the primary driver. However, to create a nutrient-limiting environment, it is more important to add proportionally more carbon than nitrogen to the soil. Otherwise, the soil microbes can utilize nitrogen from the amendment source
thus leaving more nitrogen available in the soil. These results are consistent with the literature. Nitrogen immobilization is a microbially mediated process, occurring when nitrogen is replaced by carbon as the limiting nutrient for microbial community growth during substrate decomposition (Barrett and Burke, 2000; Manzoni et al., 2008). It is well established that a substrate’s C:N is a critical determinant of the rate and duration of nitrogen immobilization or mineralization processes (Hodge et al., 2000; Manzoni et al., 2010); however there are exceptions to this trend (Hättenschwiler et al., 2011).

Future research should focus on how to refine nitrogen immobilization into a precise reverse fertilization tool. In the current study, large quantities of amendments were added to the soil to dramatically increase soil carbon, with little regard for decomposition rates. Reverse fertilization, however, may be more effective if little to no nitrogen is available for plants early in the growing season assuming the soil quickly transitions to nitrogen mineralization once crops have become established. The period of immobilization should correspond with the duration of time that nutrient resources are available in the crop seed, allowing the crop to establish dominance over the weed community before soil nitrogen becomes more readily available. This timeframe would be crop specific and may be similar to the critical period of weed control. Understanding immobilization and mineralization rates as well as amendment traits will be crucial to accurately model and time immobilization caused by different amendments so that the ideal amendment can be selected. Many studies have suggested that organic matter characteristics, in addition to C:N, affect the rate and duration of nitrogen immobilization or mineralization. Lignin content (Aber and Melillo, 1982) and solid state $^{13}$C NMR spectroscopy (Bonanomi et al., 2013) have been found to be useful in
predicting substrate decomposition rates. Bonanomi et al. (2019) suggest a framework dividing amendments into four quadrants based on two scales: carbon complexity/availability and nitrogen content. High C:N amendments with low-complexity carbon may be more appropriate for reverse fertilization.

4.5.3 Broad measures of soil biological health drive improvements in carbon amended soil

Stimulating microbial growth with high carbon amendments and altering microbial community composition was expected to have multifunctional benefits for improved soil health. A main tenet of the soil health paradigm is to recognize the living component of soil and the important functions of soil biota in nutrient retention and cycling (Haney et al., 2018; Stika, 2013; Chaparro et al., 2012). Many biological soil health indicators are measures of soil carbon and carbon cycling (Bünemann et al., 2018; Liptzin, 2022). Soil carbon is the primary driver of the soil food system and is therefore critically important in increasing soil health (Lal, 2014, 2016). Consistent with this principle, we found that soil health ratings increased in our sawdust-amended plots and sawdust and rye hay combined plots, which had high amounts of carbon added to the soil.

Increased soil health ratings in plots with high rates of carbon amendment were primarily driven by biological soil health indicators such as soil organic matter, ACE soil protein index, and active carbon. Both organic matter and active carbon are primarily measures of soil carbon. Active carbon is a measure of the potassium permanganate oxidizable carbon, which is a proxy for the food source of microbial communities (Weil et al., 2003). The autoclaved-citrate extractable (ACE) soil protein
index estimates the amount of the organically bound, or bioavailable, nitrogen by measuring a range of proteins extracted from soil (Hurisso et al., 2018). All of these measures were highest in sawdust and rye hay combined soils, which were the soils amended with the most carbon and with the greatest microbial respiration. It is logical that these indicators are increasing together, as more microbial energy sources are added to the soil.

Increased soil health ratings in our study were also associated with chemical soil health indicators: soil moisture, phosphorous, potassium, calcium, copper, magnesium, manganese, and zinc. Soil moisture may have increased due to the increased water-holding capacity in soils with added organic matter (Thangarajan et al., 2013). Increases in macro- and micro- nutrient availability may have come from the added sawdust or rye hay (Sadeghpour et al., 2021), or increased microbial activity may have improved access to soil nutrients (Song et al., 2019).

These improved soil biological health indicators were well correlated with the amount of total carbon added to the soil. However, even though microbial beta diversity was significantly affected by soil amendment treatment (Table 6), community composition clustered more strongly around rye hay. Rye hay-amended plots did seem to support the growth of beneficial microbial communities, but that change was not captured in the soil health indicator tests. This is consistent with the literature, where it is often difficult to directly connect the whole soil microbiome to soil health functioning (Bunemann et al., 2018). The measures of soil biological health often included in soil health studies are primarily measures of carbon and nitrogen cycling, which are indirect measures of the soil microbial community. It is still expensive to measure the microbial
community composition directly and more research needs to be done to link specific communities to improved soil health. A recent study by Wilhelm et al. (2022) used the microbial community composition to predict soil health ratings with almost 70% accuracy. In our study the total microbial activity was more important for improving soil health ratings and altering plant growth dynamics than the specific microbial community composition.

4.5.4 Soil amendments, especially the presence of rye hay, drove microbial community composition

In this study we consistently observed microbial community selection based on amendment treatment for both bacteria and fungi each year (Table 6). However, amending plots with rye hay had even stronger selective effects on microbial community composition than total carbon or C:N. Comparisons between rye hay-amended plots (including both rye hay plots and sawdust and rye hay combined plots) and no-rye plots (including both unamended controls and sawdust plots) showed clearer patterns than comparisons among all five amendment treatments. Although amendment treatment was significantly associated with bacterial and fungal beta diversity, the random forest model was not accurate when separating soils by amendment treatments. However, the random forest model was highly accurate in separating soils into rye hay or no-rye hay. For both bacteria and fungi in each year, differentially abundant OTUs were identified by Maaslin2 but not ALDEx2. ALDEx2 is more conservative in measuring differential abundance than Maaslin2; however, Maaslin2 is more conservative than other methods (Nearing et al., 2022). Combining methods (Maaslin2 and random forest) can increase
confidence in the conclusion that there is a strong difference in microbial community between rye versus no-rye plots.

Rye hay is notable in that it can result in allelopathic interactions with other plants. Many allelochemicals are found in rye plants but benzoxazinones may be the primary compound responsible for allelopathy (Schulz, 2013). They are released as root exudates or during residue decomposition and are most abundant in young leaves (Schulz, 2013; Du Fall and Solomon, 2011). Benzoxazinoids disrupt many cellular activities by intercalating with nucleic acids and interacting with proteins (Du Fall and Solomon, 2011), thereby inhibiting germination, and reducing seedling growth, especially growth of root tips (Schulz, 2013). Both the allelopathic compounds and the breakdown products of these compounds can also affect soil microbial growth (Du Fall and Solomon, 2011) and may be responsible for the large microbial response we observed in rye-amended soils.

Although allelopathic compounds have been linked to specific soil microbes, there has often been a poor correlation between rye and whole soil microbiomes in the literature. Several studies found no effect of rye cover crops on soil microbial communities compared with other cover crop species (Arenz et al., 2014; Chamberlain et al., 2020). Other studies reported small differences in microbial community characteristics in the presence of a rye cover crop, such as increased fungal abundance (Li and Wu, 2018) and increased alpha diversity of bacterial communities relative to no cover crop (Fernandez et al., 2016). Tyler (2021) found no significant differences in bacterial community composition between rye and clover cover-cropped soils at a high taxonomic level but did find that rye cover-cropped soils had higher levels of
Bradyrhizobium than soils without cover crops. Similarly, Patkowska et al. (2016) found that rye cover-cropped soils had the greatest total population of bacteria and increased abundance of Bacillus spp. and Pseudomonas spp.

The inconsistent effect of rye on the soil microbiome in the literature makes the strong effect of rye amendments in our study somewhat surprising. There may be several reasons for the more pronounced differences in our experiment. We were comparing amendment types and crop species within one field, reducing edaphic soil variability (Arnez et al., 2014). We also added a large amount of young rye. Most cover crop experiments utilize older rye, which produce fewer allelopathic compounds (Schulz, 2013). Additionally, we incorporated rye into the soil, which may have increased the influence of rye in our study compared with other cover crop experiments, which typically leave rye residue on the soil surface (Pascault et al., 2010).

Exploring the differentially abundant OTUs based on rye amendment in our experiment, many of the microbes that were more abundant in rye-amended soils appear to be beneficial. Two strains of Stenotrophomonas geniculata were significantly more abundant in rye-amended soils, compared with no-rye soils, in 2020. Stenotrophomonas geniculata has been shown to have plant growth-promoting properties in chickpea nodules and corn (Gopalakrishnan et al., 2015; Singh et al., 2020). Stenotrophomonas geniculata can also decompose complex compounds such as the herbicide, paraquat (Wu et al., 2020), nicotine (Liu et al., 2014), and polybutylene succinate/polylactic acid (Srimalanon et al, 2020). Pseudomonas vancouverensis was also more abundant in rye-amended soils and has been shown to improve the stress tolerance of red peppers (Capsicum annuum L.) (Samaddar et al. 2019) and tomatoes (Solanum lycopersicum L.)
An uncultured Staphylococcus, which was more abundant in rye hay plots than no-rye plots, was the only OTU shared between the 2020 and 2021 lists of the top 10 bacterial OTUs with significantly different abundance. In 2021, the three most significantly different bacteria were Bacillus species, which contribute to macronutrient cycling by solubilizing phosphorous-containing potassium compounds (Song et al., 2018). These three bacteria were more abundant in rye hay-amended soils, although the 6th most significantly different OTU was a Bacillus species more abundant in no-rye soils.

Two fungal OTUs were identified by Maaslin2 as significantly different between rye hay and no-rye plots in both years. Both OTUs were more abundant in rye-amended soils. Papiliotrema laurentii is found in a variety of habitats and can metabolize a variety of carbon substrates. It can control phytopathogenic fungi and improve mycorrhizal colonization, nitrogen retention, and plant growth (de Almeida et al., 2022). The other fungus was in the family Nectriaceae, but not further classified. Trichsporon insectorum, Ascobolus foliicola, and Trichoderma evansii were all more abundant in rye-amended soils in 2021. Trichsporon insectorum is a yeast, often associated with insects, with fungicidal properties (Fuentefria et al., 2008). Ascobolus foliicola is often found on bare ground or decomposing plant matter (Uzun et al., 2017) and Trichoderma evansii was isolated from the sapwood of Lophira alata and Cola verticillate (Samuels and Ismaiel, 2009).

Although allelopathic compounds can alter microbial and weed community composition, this factor likely did not influence weed responses in our study. While rye hay contributes to weed suppression through several mechanisms (Barnes and Putnam,
1983), we observed a stronger effect of nitrogen availability on weed biomass. Weed biomass in rye hay-amended plots increased over the nontreated control, indicating that an increase in nitrogen and beneficial microorganisms were more beneficial to weed biomass than allelopathic compounds were detrimental. In our study the greatest reduction in weed species biomass was in sawdust-amended plots, which had high nitrogen immobilization but no allelochemicals.

4.5.5 Conclusion

In summary, this study demonstrates that reverse fertilization can improve the competitive advantage of leguminous crops with multifunctional benefits for soil health. High C:N organic amendments stimulate nitrogen immobilization through microbial growth and greater amounts of carbon added to the soil improved soil health ratings. The soil microbial community composition consistently changed when different amendments were added to the soil; however, these compositional shifts were most closely associated with the presence of rye hay. Though these results are promising, reverse fertilization requires further refinement before it can be adopted by growers. Potential ways to refine this tool include immobilizing nitrogen only in specific locations such as between the crop rows or at the soil surface to inhibit the germination of small-seeded, r-selected weeds. Alternatively, nitrogen may be immobilized only during specific times, such as within the critical period of weed control, or for a few years before competitive species begin to dominate. Nitrogen immobilization is an important regulator of plant growth that should be considered when designing weed management strategies in agroecosystems.
REFERENCES

Abarenkov, K., Henrik Nilsson, R., Larsson, K.-H., Alexander, I.J., Eberhardt, U., 
Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, 
A.F.S.,Tedersoo, L., Ursing, B.M., Vrålstad, T., Liimatainen, K., Peintner, U., 
https://doi.org/10.1111/j.1469-8137.2009.03160.x

litter as a function of initial nitrogen and lignin content. Can. J. Bot. 60, 2263– 
2269. https://doi.org/10.1139/b82-277

https://doi.org/10.1038/s41893-019-0415-y

Papiliotrema Laurentii: General features and biotechnological applications. 
Appl Microbio and Biotech 106, 6963–6976. https://doi.org/10.1007/s00253- 
022-12208-2

Arenz, B.E., Bradeen, J.M., Otto-Hanson, L.K., Kinkel, L.L., 2014. Two grass species 
fail to display differing species-specific effects on soil bacterial community 
https://doi.org/10.1007/s11104-014-2226-2


https://doi.org/10.1614/WS-08-065.1


Bonanomi, G., Sarker, T.C., Zotti, M., Cesarano, G., Allevato, E., Mazzoleni, S., 2019. Predicting nitrogen mineralization from organic amendments: beyond
C/N ratio by 13C-CPMAS NMR approach. Plant Soil 441, 129–146.  
https://doi.org/10.1007/s11104-019-04099-6


experiments by compositional data analysis. Microbiome 2, 15.

https://doi.org/10.1186/2049-2618-2-15


https://doi.org/10.1007/s00253-016-7736-9


https://doi.org/10.1038/s42003-022-03860-5


Garnier, E., Cordonnier, P., Guillerm, J.-L., Sonié, L., 1997. Specific leaf area and leaf nitrogen concentration in annual and perennial grass species growing in


191


https://doi.org/10.1038/s43017-020-0080-8


https://doi.org/10.1007/s11104-008-9568-6


Samaddar, S., Chatterjee, P., Roy Choudhury, A., Ahmed, S., Sa, T., 2019. Interactions between Pseudomonas spp. and their role in improving the red


https://doi.org/10.2489/jswc.68.4.99A


https://doi.org/10.1017/S0043174500057568


Wallingford, UK: CABI


https://doi.org/10.1007/BF00010918


201


https://doi.org/10.2134/agronj2012.0396


https://doi.org/10.1614/WS-D-10-00089.1


Appendix Table 1.1. Summarized results from restoration ecology literature on carbon amendments as a tool to manage invasive plants. Invasive species names are shown in bold text.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Summary</th>
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<tbody>
<tr>
<td>McLendon &amp; Redente, 1992</td>
<td>Carbon-amended treatments had significantly higher species richness, more perennial forbs, more annual grasses, higher <em>Machaeranthera asteroids</em> (Torr.) Greene canopy cover, fewer annual forbs, and marginally less <em>Kochia scoparia</em> (L.) A.J. Scott canopy cover than nitrogen-amended treatments. The nitrogen contents of <em>Salsola tragus</em> L. and <em>Kochia scoparia</em> (L.) A.J. Scott were lowest in carbon-amended treatments and highest in nitrogen-amended treatments. The nitrogen content of <em>Ericameria xbolanderi</em> (A. Gray) G.L. Nesom &amp; Baird was not affected by soil amendments but was higher than the nitrogen contents of all other plant species in carbon-amended treatments.</td>
</tr>
<tr>
<td>Morgan, 1994</td>
<td>Carbon-amended treatments caused a dramatic visible reduction in weed growth without hindering native species growth at one of three sites, but did not have this effect at the other two sites.</td>
</tr>
<tr>
<td>Young et al., 1998</td>
<td>Carbon-amended treatments had less <em>Taeniatherum caput-medusae</em> (L.) Nevski growth and seed production at all moisture levels, but viable seeds were able to accumulate in the seedbank. Native grasses did not reestablish despite reduced <em>Taeniatherum caput-medusae</em> (L.) Nevski growth.</td>
</tr>
<tr>
<td>Zink &amp; Allen, 1998</td>
<td>Carbon-mulched plots had higher <em>Artemisia californica</em> Less. seedling survival than unmulched plots in the first year. Mulched plots had higher <em>Artemisia californica</em> Less. volume by the second year and this trend remained significant over time.</td>
</tr>
<tr>
<td>Reever Morghan &amp; Seastedt, 1999</td>
<td>Carbon-amended plots had significantly less available soil nitrate during treatments, but the difference in available nitrate between carbon and unamended plots decreased with time. Total plant biomass was reduced in carbon-amended treatments compared to unamended treatments, regardless of whether the plant species was native. There was no difference in seed number per gram of <em>Centaurea diffusa</em> Lam. tissue biomass.</td>
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<tr>
<td>Paschke et al., 2000</td>
<td>Carbon-amended treatments had an increase in the abundance of perennials, relative to annual grasses and forbs, and less inorganic nitrogen. Nitrogen-amended treatments had an increase in the biomass of annual grasses and forbs, relative to perennials, and more inorganic nitrogen.</td>
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<td>Alpert &amp; Maron, 2000</td>
<td>Carbon-amended treatments had 40% less non-native plant biomass than unamended treatments after two years. This effect was driven primarily by non-native grasses.</td>
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<td>Blumenthal et al., 2003</td>
<td>Carbon-amended treatments had less available nitrogen when more carbon was applied, but microbial biomass did not always increase. Plant communities were not analyzed.</td>
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<td>Corin and D’Antonio, 2004</td>
<td>Carbon-amended soil had reduced growth of <em>Nassella pulchra</em> (Hitchc.) Barkworth, relative to unamended soil, in the absence of annual exotic species. Under competition from annual exotic species, carbon-amended soil planted plots had a direct effect on the growth of <em>Nassella pulchra</em>. The results suggest that carbon amendments can be an effective tool for managing invasive plants.</td>
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</table>

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had increased growth of *Nassella pulchra* (Hitchc.) Barkworth, relative to unamended soil. There was no effect of carbon in the second growing season.

<table>
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<tr>
<th>Reference</th>
<th>Summary</th>
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<tbody>
<tr>
<td>Perry et al., 2004</td>
<td>In carbon-amended soil, <em>Phalaris arundinacea</em> L. biomass was reduced by 82% when grown in competition with <em>Carex hysterica</em> Muhl. ex Willd but <em>Carex hysterica</em> Muhl. ex Willd biomass was only reduced by 32% when grown in competition with <em>Phalaris arundinacea</em> L.. In nitrogen-amended soil, <em>Carex hysterica</em> Muhl. ex Willd. biomass was reduced by 91% when grown in competition with <em>Phalaris arundinacea</em> L., but <em>Phalaris arundinacea</em> L. was unaffected by <em>Carex hysterica</em> Muhl. ex Willd.</td>
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<td>Baer et al., 2004</td>
<td>Carbon-amended treatments had the lowest non-native and total plant cover in year one, but an increase in species richness over time. Carbon-amended treatments had the highest percent cover of <em>Sorghastrum nutans</em> (L.) Nash and <em>Schizachyrium scoparium</em> (Michx.) Nash but the lowest percent cover of <em>Panicum virgatum</em> L., which was inversely related to plant diversity. Nitrogen-amended treatments had a decrease in species diversity and richness, but the highest native species cover by year three. Carbon-amended treatments had a species composition that was more similar to the native prairie species composition (as opposed to restored prairie) than all other treatments.</td>
</tr>
<tr>
<td>Huddleston &amp; Young, 2005</td>
<td>Carbon-amended treatments reduced soil nitrogen availability but had no significant effect on plant cover, relative to the control. Herbicide applications did improve native plant density, cover, and biomass.</td>
</tr>
<tr>
<td>Prober et al., 2005</td>
<td>Carbon-amended treatments had reduced biomass of exotic annuals and increased abundance of native perennial grasses: <em>Themeda triandra</em> Forssk, when it was seeded, or <em>Austrostipa bigeniculata</em> (Hughes) S.W.L. Jacobs &amp; J. Everett, <em>Bothriochloa macra</em> (Steud.) S.T.Blake and <em>Aristida ramosa</em> R.Br. in unseeded plots. Re-established <em>Themeda triandra</em> Forssk further reduced soil nitrate.</td>
</tr>
<tr>
<td>Eschen et al., 2007</td>
<td>Carbon-amended treatments had an overall reduction in plant biomass the first year in Switzerland and the second year in the UK. They consistently increased bare ground, even after treatments were stopped. After the second treatment year, carbon-amended treatments in Switzerland had increased relative cover of legumes, forbs, and late seral species and carbon-amended treatments in the UK had reduced relative cover of bryophytes.</td>
</tr>
<tr>
<td>Mazzola et al., 2008</td>
<td>Carbon-amended treatments reduced <em>Bromus tectorum</em> L. biomass, density, and seed production and <em>Agropyron fragile</em> [Roth] P. Candargy growth in the first year, but these effects were not observed in the second year.</td>
</tr>
<tr>
<td>Brunson et al., 2010</td>
<td>Carbon-amended treatments reduced the growth of the entire ruderal plant community, not just the invasive annual grass community. As more carbon was added, nitrate became less available and microbial biomass increased, but this effect was short-term.</td>
</tr>
<tr>
<td>Yelenik et al., 2016</td>
<td>Carbon-amended treatments had no effect on survivorship and a negative effect on growth of all outplanted species, regardless of nitrogen-fixing status. Nitrogen-amended treatments had reduced survival of both nitrogen-fixing species, <em>Morella faya</em> (Aiton) Wilbr (Myricaceae) and <em>Acacia koa</em> A. Gray, and greater cover and height of the perennial grass <em>Melinis minutiflora</em> P.</td>
</tr>
<tr>
<td>Morris &amp; Gibson-Roy, 2018</td>
<td>Carbon-amended treatments had reduced re-growth of total, native, and exotic canopy abundance. Treatments combining low rates of carbon amendment with fire increased native species richness but did not affect exotic species richness compared with untreated plots. High rates of carbon-amendment reduced native species richness.</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Summary</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Brown et al., 2017</td>
<td>Carbon-amended treatments decreased weed emergence more than glyphosate or scalping alone. However, scalping alone had the most success in native plant recruitment (29 recruits m⁻²) compared to all other treatments.</td>
</tr>
<tr>
<td>Busby et al., 2019</td>
<td>Carbon-amended treatments had equal or less total basal vegetation and weed cover, relative to unamended treatments, at both sites. Relative planted grass shoot cover increased at one site. Soil physical and chemical characteristics improved at the other site.</td>
</tr>
<tr>
<td>Uddin et al., 2020</td>
<td>Carbon-amended treatments led to greater total nitrogen reduction in <em>Phragmites australis</em> (Cav.) Trin ex Steud. than <em>Melaleuca ericifolia</em> Sm., reduced <em>Phragmites australis</em> (Cav.) Trin ex Steud. growth by 39% (uncut) or 57% (repeated cut), and increased <em>Melaleuca ericifolia</em> Sm. growth by 41% (uncut) or 68% (repeated cut).</td>
</tr>
<tr>
<td>Cole et al., 2021</td>
<td>Carbon-amended treatments had less available soil nitrogen in both the tropical wet forest and the dry shrubland ecosystems. <em>Cheirodendron trigynum</em> (Gaudich.) A. Heller seedling survival was higher in carbon-amended treatments than fertilized treatments. Change in heights for both <em>Psidium cattleianum</em> Sabine and <em>Chenopodium oahuense</em> (Meyen) Aellen was higher in nitrogen-amended treatments than other treatments. Both <em>Senecio madagascariensis</em> Poir. (fourfold) and <em>Eragrostis atropioides</em> Hillebr. (twofold) had an increase in reproductive output in nitrogen-amended treatments.</td>
</tr>
<tr>
<td>Knauf et al., 2021</td>
<td>Carbon-amended treatments had reduced <em>Pennisetum setaceum</em> (Forsk.) Chiov inflorescences, decreased <em>Dodonaea viscosa</em> (L.) Jacq. growth, and all invasive and resource exploitative species had decreased photosynthetic nitrogen. <em>Metrosideros polymorpha</em> Gaud. had greater seedling survival and the growth of other native species was unaffected in carbon-amended treatments.</td>
</tr>
</tbody>
</table>
Appendix Figure 2.1. Heatmap showing relative abundance of bacterial (A) and fungal (B) operational taxonomic units (OTUs) from pots filled with high-carbon-amended or unamended soils. The OTUs shown are the 20 most predictive of soil amendment type based on p-values calculated from Maaslin2. The phylogenetic order and class (for bacteria and fungi, respectively) and p-value of each OTU is shown to the right. Most of the OTUs are more abundant in carbon-amended soil than in unamended soil for both bacteria (17 of 20) and fungi (16 of 20). The 6-digit identifiers of each OTU correspond to the same 6-digit identifier in Table 2.3.
**Appendix Table 4.1.** Baseline soil nutrient levels in 2018 for the corn and soybean fields amended with rye hay and sawdust in Freeville, NY

<table>
<thead>
<tr>
<th>Soil nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter (%)</td>
<td>2.74</td>
</tr>
<tr>
<td>pH</td>
<td>6.27</td>
</tr>
<tr>
<td>P (mg·Kg⁻¹)</td>
<td>20.4</td>
</tr>
<tr>
<td>K (mg·Kg⁻¹)</td>
<td>100.8</td>
</tr>
<tr>
<td>Al (mg·Kg⁻¹)</td>
<td>24.6</td>
</tr>
<tr>
<td>Ca (mg·Kg⁻¹)</td>
<td>1053</td>
</tr>
<tr>
<td>Fe (mg·Kg⁻¹)</td>
<td>2.0</td>
</tr>
<tr>
<td>Mg (mg·Kg⁻¹)</td>
<td>63.1</td>
</tr>
<tr>
<td>Mn (mg·Kg⁻¹)</td>
<td>14.9</td>
</tr>
<tr>
<td>Zn (mg·Kg⁻¹)</td>
<td>19.6</td>
</tr>
</tbody>
</table>

**Appendix Figure 4.1.** Accumulated rainfall at the Freeville research farm in 2020 (orange line), 2021 (blue line), and average over 10 years from 2010-2019 (black line). Shaded area is the standard error of the mean.