Micro-managing soil health: leveraging plant-microbe interactions to improve the effectiveness of cover cropping strategies (Ceres 2015-01667)

Final Research Report
March 2018

Project Objectives

The goal of this research project was to improve cover-cropping strategies in organic agriculture by understanding how cover crops interact with soil microorganisms to affect soil health. To meet this goal, we sought to address the following specific objectives: 1) determine how different cover crops shape soil microbial community composition; 2) identify nitrogen-sequestering and nitrogen-releasing microbes in cover crop systems and determine how their activity changes following cover crop termination; 3) identify weed-suppressive microbes in cover crop systems and determine how their activity changes following cover crop termination.

Our research program included both field-based experiments on working organic farms and controlled laboratory experiments to characterize different aspects of soil health. We used these two different experimental platforms to generate a large set of samples that we further characterized by classical soil chemistry techniques and cutting-edge, DNA-based approaches to soil microbial community ecology. Our methods are more fully documented in Section 5 of the research proposal.

Research Performed

This project entailed two years of field work on two different certified organic farms, and this was followed by a year of more intensive, laboratory-based research. I have provided detailed descriptions of the work accomplished in 2015 and 2016 in my two previous interim reports, and I refer you to those reports for a more information on these first two project years. What follows is a brief summary of the research performed in the first two project years, followed by a more detailed description of the research from the final project year.

In 2015 and 2016 we collected soil samples from spring cover crop trials established on two organic farms as part of a separate, but related, Ceres-funded project (Wortman 2013, Keystone cover crop species: understanding the relative contribution of individual species to soil health). We quantified *weed-suppressiveness* of these soils using seed germination bioassays. We also extracted microbial community DNA, inorganic nitrogen, and potentially mineralizable nitrogen from these soils in order to understand how cover crops and their residues affected the *soil microbial community*, and *nitrogen dynamics* following cover crop termination. These research activities followed the procedures outlined in our proposal, with the following important differences:

(1) We sampled each of these plots on three separate occasions following cover crop termination: within 1 week of termination; approximately two weeks after termination; and approximately four weeks after termination. We reduced the number of sampling events (from 5 time points in the proposal) because of poor weather and because of concerns about soil disturbance from repeated collections We were able to successfully collect our three target time points at each farm in both years.

(2) At one of our farms (Prairie Earth Farm), we established our spring cover crop trials in a section of field that had formerly contained an oat + field pea cover crop. Unfortunately, after establishing our experiment, we discovered that the former cover crop had not been completely killed off, and we found that all of our plots contained oats and field peas. It was not possible to distinguish these "volunteer" plants from those that we planted. Because accurate knowledge of the seeding rate is an important factor in collaborator Wortman's experiment, we decided to discontinue most of the plots in this particular trial. However, we decided to continue soil health monitoring in four plots (mustard, purple top turnips, weedy control, and weed-free control) in order to follow up on some patterns that we identified in the 2015 soil data.

According to the research plan from our proposal, we did not conduct any additional field work in 2017, as 2016 was the final field season planned in the Wortman 2013 project. Instead, we focused on three additional analyses of the soils that we collected in 2015 and 2016. We quantified *microbial biomass carbon* and *microbial biomass nitrogen* in all of our soils using a chloroform-fumigation technique. We also quantified *soil phenolic carbon* concentration, as previous work has found that soil phenolic compounds can be used as proxies for bioactive compounds (e.g. allelochemicals) released by some cover crops during their active phases and after termination. Finally, we carried high-throughput DNA sequencing of *soil microbial communities* in order to understand how cover crops can change the structure and function of soil communities.

Results of Research Activities

We found that soil nitrate, soil ammonium, and potentially mineralizable nitrogen varied in each of our four site-years (two farms x two years), and that these soil pools were also significantly different at different sampling times following cover crop termination (Tables 1 and 2). In general, we found that soil ammonium and nitrate values were highest in the sample collected two weeks after termination (Figs. 1 and 2), suggesting that decomposition of cover crop residues results in a "pulse" of inorganic nitrogen released to the soil at this time. In contrast, potentially mineralizable nitrogen was highest in the final soil sample (Fig. 3), suggesting that organic nitrogen continues to be a potential source of fertility for plants up to 4 weeks following termination. We also found significant differences in soil nitrate and potentially mineralizable nitrogen content across our different cover crop treatments (Table 1), but these differences were generally due to the fact that soil

nitrogen was substantially different in our plant-free (i.e. no cover crops, weeds removed) treatment as compared to all other plots.

To better understand how cover crops affected the dynamics of soil nitrogen, we used a basic machine-learning approach called Classification and Regression Trees (CART). CART used information on the biomass of all cover crops in the plots at termination, in conjunction with the number of days since cover crop termination. This allowed us to classify soil samples into those with high pools of nitrogen vs. those with low pools of nitrogen. By following the pathway of explanatory variables from the top of the CART diagram down to the bottom of the diagram, we can see how cover crop performance interacted over time to influence different nitrogen pools.

These CART analyses revealed a very strong effect of Year on soil nitrate and ammonium levels. Soil nitrate was higher at both farms in 2015 compared to 2016 (Fig. 4), with the highest soil nitrate levels observed within 1 week of termination at PrairiErth Farm in 2015 (Fig. 4; node # 15). Soil ammonium levels also tended to be higher in 2015 than 2016 (Fig. 5; nodes #2 and 3), but soil ammonium was dynamically different in these two years. In 2016 we observed the highest soil ammonium levels in plots with lots of mustard biomass at termination (Fig. 5, node #5), while in 2015 the soil ammonium levels changed primarily with time following termination (Fig. 5; nodes #6, 7, and 13) or in plots that had high plant biomass (of any kind) at termination (Fig. 5; node # 25). In contrast to these inorganic nitrogen pools, we found that potentially mineralizable nitrogen was very responsive to the amount of plant biomass in the plots at cover crop termination (Fig. 6). Of particular note, we found that high mustard biomass generally associated with the highest levels of potentially mineralizable nitrogen (Fig. 6; nodes #30, 62, 63).

We found that soil phenolic content—a rough proxy for allelochemical compounds that can be released by cover crops upon termination—was significantly higher in 2016 than in 2015 (Tables 1 and 2). Soil phenolic content tended to increase over time following cover crop termination (Table 1, Fig. 7). We did not find any significant effects of cover crop type on soil phenolic content (Table 1), which is surprising, given that mustard cover crops and legume cover crops have been shown release allelochemicals to the soil upon termination.

We found different levels of weed suppressiveness these cover-cropped soils, but no particular cover crop species was clearly the best weed suppressor at both sites or even over time. As an example, we present the results of our 2016 bioassays here (we have previously presented these results in the 2016 interim report). In 2016, the germination rates of lettuce, velvetleaf, and field pennycress changed over time, but they showed opposing patterns at each of our two organic farms (Figures 8 and 9). At Prairie Earth Farm, all three plants experienced their lowest germination rates in soils collected 33 days after cover crop incorporation (Figure 8). In contrast, we found the lowest germination rates of plants in the soils collected within 5 days

of cover crop incorporation at Kinnikinnick Farm (Figure 9). We also found that different cover crops suppressed seed germination to different extents (Figures 10 and 11). At Prairie Earth Farm, we found that field pennycress germinated best in soils that had no plant cover (WF treatment, Figure 10), with the native weed community being just a suppressive of field pennycress as either mustard or purple top turnips. Interestingly, the native weed community suppressed lettuce germination more than mustard or turnips (Figure 10), while mustard produced the highest germination rates of velvetleaf (Figure 10). Our data from Kinnikinnick Farm suggest that field peas and faba beans may have a negative impact on lettuce germination (Figure11). We found little evidence that mustard suppressed seed germination rates at Kinnikinnick Farm (Figure 11).

We found no significant patterns of microbial biomass carbon or microbial biomass nitrogen with respect to site, year, time, or cover crop treatment (data not shown). However, our numbers for microbial biomass carbon and nitrogen were substantially lower than what is often reported in the literature for agricultural soils, and so we suspect that our analytical technique was not very reliable.

Altogether, we produced 21,685,014 high-quality DNA sequences from these soils, and we used bioinformatics analysis of these sequences in order to identify the bacteria and fungi present in our soils. Based on these analyses, we identified 16,062 unique bacterial species and 4,925 fungal species. We refer to these species as "operational taxonomic units" (OTUs), because our species identifications are based on DNA sequences of organisms that we have not been able to cultivate in the laboratory, and these kinds of bioinformatics identifications are regarded as provisional. Nevertheless, this very large DNA sequencing study allowed us to determine that soil microbial communities were significantly different at each of our farms, and they changed significantly over time (year and days after cover crop termination; Table 3). Furthermore, we found that both bacterial and fungal communities were different in cover crop mixtures than in cover crop monocultures (Table 3), and fungal communities differed significantly between the different cover crop treatments.

We used a multivariate data analysis technique called partial least squares regression (PLSR) to look for significant relationships between specific cover crop plants (based on their biomass at the time of termination) and specific microbial species (OTUs). Our PLSR analysis revealed that two cover crop species, mustard and oats, were the strongest influencers of microbial community composition in soils (Table 4), although the biomass of weeds was also a consistent influencer of microbial communities (Table 4). PLSR helped us identify a subset of microbes that are strongly associated (either positively or negatively) with each of these influential cover crop species (Tables 5-8). Of particular note for the objectives of this project, we found that soil Archaea in the genus *Nitrososphaera* were commonly identified as associates of these cover crops (Tables 5 and 6). These are nitrifying

organisms that convert ammonium to nitrate in the soil, and so they are key nitrogen cycling organisms in the soil.

Significance of the Research

While the relationship between cover crops and soil health is complex, our research has demonstrated several important features of cover cropping systems. We have shown that cover crop residues can be a continuing source of nitrogen to the soil following termination. Pulses of inorganic nitrogen are delivered to the soil roughly two weeks following termination, and further mineralization of organic nitrogen from cover crop residues can provide additional nitrogen for at least four weeks following termination. The amount of nitrogen delivered in our trials was strongly dependent on the biomass of plant material present in our plots at the time of termination, and the most influential cover crops (generally mustard and oats) were also those that tended to be the most productive species in mixtures and monocultures. Thus, soil health benefits from cover crops will be maximized when cover crops establish well and are able to accumulate significant biomass before they are terminated. In addition, these productive cover crops (mustard and oats) can alter the community composition of key nitrifying organisms in the soil, and so they can impact the rates of nitrogen transformation and retention in soils.

Table 1. Results of fully factorial ANOVA tests on measures of soil nitrate, ammonium, PMN and phenolic content by site, year, cover crop treatment and sample date. Only significant interactions ($p < 0.05$) are reported. Significant effects are labeled with an asterisk. All significant results were followed by Tukey HSD post-hoc test ($p < 0.05$). df = degrees of freedom: numerator, total; F = F statistic; p = p-value; effect size = sum of squares (predictor)/sum of squares (total).

Soil quality measure	*Source*	*df*	*F*	*p*	*Effect size*
Nitrate	Site	1,425	263.53	<0.0001*	0.620
	Year	1,425	454.65	<0.0001*	1.070
	Cover crop treatment	13,425	2.05	0.016*	0.063
	Sample date	2,425	49.55	<0.0001*	0.233
	Site x cover crop treatment	13,425	2.13	0.012*	0.065
	Site x Sample date	2,425	39.97	<0.0001*	0.188
	Year x Sample date	2,435	36.14	<0.0001*	0.170
	Site x Year x Sample date	1,425	6.58	0.011*	0.015
Ammonium	Site	1,420	50.57	<0.0001*	0.120
	Year	1,420	57.46	<0.0001*	0.137
	Cover crop treatment	13,420	0.466	0.943	0.014
	Sample date	2,420	13.87	<0.0001*	0.066
	Site x Sample date	2,420	50.54	<0.0001*	0.241
	Year x Sample date	2,430	20.74	<0.0001*	0.099
	Site x Year x Cover crop treatment	3,420	2.88	0.036*	0.021
	Site x Cover crop treatment x Sample date	16,420	2.05	0.010*	0.078
PMN	Site	1,424	26.14	<0.0001*	0.062
	Year	1,424	55.22	<0.0001*	0.130
	Cover crop treatment	13,424	2.98	0.0003*	0.091
	Sample date	2,424	5.37	0.005*	0.025
	Site x Year	1,424	8.55	0.004*	0.020
	Site x Sample date	2,424	3.93	0.020*	0.019
	Year x Sample date	2,424	15.53	<0.0001*	0.073
	Site x Year x Cover crop treatment	3,424	4.70	0.003*	0.033
	Site x Year x Sample date	1,424	12.39	0.0005*	0.029
Phenolic content	Year	1,76	24.44	<0.0001*	0.322
	Cover crop treatment	3,76	2.18	0.098	0.086
	Sample date	2,76	9.360	0.0002*	0.246

Table 2. Means and standard deviations of nitrate, ammonium, PMN and soil phenolic content. Two-way ANOVA tests revealed effects of site and year on these measures, so the data was divided into four site-year combinations (Table 1). Letters indicate significant differences within each individual measure (Tukey's HSD post-hoc test, $p < 0.05$).

Site and year	Nitrate (mg NO_3-N/kg)	Ammonium (mg NH_4-N/kg)	Potentially mineralizable N (mg NH_4-N/kg)	Phenolic content (mg gallic acid eq./L)
PrariErth 2015	21.06 ± 7.24^{a}	7.14 ± 1.44^{a}	73.14 ± 14.37^{ab}	0.85 ± 0.34^{a}
PrariErth 2016	9.78 ± 6.22^{b}	6.36 ± 3.03^{b}	69.08 ± 10.86^{bc}	1.12 ± 0.21^{b}
Kinnikinnick 2015	15.96 ± 6.21^{c}	6.61 ± 1.78^{b}	72.07 ± 25.19^{ac}	---
Kinnikinnick 2016	7.47 ± 3.10^{d}	5.44 ± 1.38^{c}	57.25 ± 12.78^{d}	---

Table 3. Permutational Multivariate Analysis of Variances tests were carried out on the entire dataset to evaluate if site, year, cover crop treatment, cover crop diversity or sample date influenced bacterial and fungal community composition. The Manhattan distance method was applied for bacterial data and the Bray-Curtis distance method was applied to fungal community data. df = degrees of freedom: numerator, total; F = F statistic; R^2 = R^2-value; p = p-value. Results were considered significant at the p = 0.05 level.

	Bacterial community composition				Fungal community composition			
Source	*df*	*F*	R^2	*p*	*df*	*F*	R^2	*p*
Site	1, 526	28.91	0.052	0.001*	1, 559	99.01	0.151	0.001*
Year	1, 526	27.91	0.050	0.001*	1, 559	41.23	0.069	0.001*
Cover crop treatment	13, 526	0.200	0.027	0.068	13, 559	1.272	0.029	0.005*
Cover crop diversity	2, 526	1.674	0.006	0.001*	2, 559	2.442	0.009	0.001*
Sample date	1, 526	2.908	0.006	0.001*	2, 559	13.37	0.046	0.001*

Table 4. Top three components from the four PLSR models. Percent variance in X (cover crop biomass) and cumulative percent variance explained by each of the four components is listed. The cover crop associated with positive or negative X loadings is also listed. Blank loading associations indicate no association with a particular species.

	Component	% variance in X explained	Cumulative % variance in X explained	Loadings
Bacteria Kinnikinnick	1	42.2%	42.2%	Mustard (-0.494), Weeds (-0.872)
	2	37.0%	79.2%	Weeds (0.541), Mustard (-0.860)
	3	8.3%	87.5%	Oat (0.989), Turnip (0.278)
Bacteria PrariErth	1	85.1%	85.1%	Mustard (-1.000)
	2	5.3%	90.4%	Weeds (1.016), Oat (-0.206)
	3	4.7%	95.1%	Oat (-1.032)
Fungi Kinnikinnick	1	42.2%	42.2%	Mustard (-0.495), Weeds (-0.872)
	2	37.1%	79.3%	Weeds (0.537), Mustard (-0.853)
	3	8.7%	88.0%	Oat (-1.005)
Fungi PrariErth	1	85.1%	85.1%	Mustard (1.002)
	2	4.9%	90.0%	Weeds (-1.054)
	3	4.8%	94.8%	Weeds (0.230), Mustard (-1.030)

Table 5. Top loading bacterial OTUs for the PLSR model at Kinnikinnick. Components correspond to the components listed in Table 4. Loadings are either the top positive ("pos") or negative ("neg") values.

Component	Bacterial OTU	Loading
1	Actinobacteria, Micrococcaceae (OTU2)	pos
	Proteobacteria, Betaproteobacteria (OTU49)	pos
	Actinobacteria, Solirubrobacterales (OTU110)	pos
	Archaea, *Candidatus Nitrososphaera* (OTU151)	pos
	Proteobacteria, Gammaproteobacteria (OTU67)	pos
1	Verrucomicrobia, [Chthoniobacteraceae], DA101 (OTU3)	neg
(Weeds, -0.872	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU66)	neg
Mustard, -0.494)	Verrucomicrobia, [Chthoniobacteraceae], DA101 (OTU11121)	neg
	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU5)	neg
	Archaea, *Candidatus Nitrososphaera*(OTU77)	neg
2	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU11804)	pos
(Weeds, 0.541)	Acidobacteria, [Chloracidobacteria] (OTU246)	pos
	Acidobacteria, [Chloracidobacteria] (OTU333)	pos
	Acidobacteria, iii1-8 (OTU306)	pos
	Chloroflexi, Gitt-GS-136 (OTU432)	pos
2	Archaea, *Candidatus Nitrososphaera*(OTU77)	neg
(Mustard, -0.860)	Gemmatimonadetes, Gemm-1 (OTU720)	neg
	Bacteroidetes, Chitinophagaceae (OTU10492)	neg
	Bacteroidetes, Cytophagaceae (BAC206)	neg
	Chloroflexi, Caldilineaceae (BAC225)	neg
3	Archaea, *Candidatus Nitrososphaera*(OTU77)	pos
(Oat, 0.989	Verrucomicrobia, [Chthoniobacteraceae], *DA101* (OTU3)	pos
Turnip, 0.278)	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU66)	pos
	Bacteroidetes, *Flavisolibacter*(OTU1849)	pos
	Gemmatimonadetes, Ellin5290 (OTU3221)	pos
3	Actinobacteria, Micrococcaceae (OTU2)	neg
	Proteobacteria, Betaproteobacteria, MND1 (OTU49)	neg
	Chloroflexi, Gitt-GS-136 (OTU432)	neg
	Acidobacteria, iii1-15 (OTU108)	neg
	Proteobacteria, Betaproteobacteria (OTU44)	neg

Table 6. Top loading bacterial OTUs for the PLSR model at PrariErth. Components correspond to the components listed in Table 4. Loadings are either the top positive ("pos") or negative ("neg") values.

Component	Bacterial OTU	Loading
1	Actinobacteria, Microbacteriaceae (OTU265)	pos
	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU35)	pos
	Archaea, Candidatus Nitrososphaera *SCA1145* (OTU6)	pos
	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU17862)	pos
	Proteobacteria, Betaproteobacteria (OTU20)	pos
1	Chloroflexi, Anaerolineae, SBR1031, oc28 (OTU4195)	neg
(Mustard, -1.00)	Bacteroidetes, Sphingobacteriales (OTU249)	neg
	Bacteroidetes, Saprospiraceae (OTU73)	neg
	Proteobacteria, Deltaproteobacteria, Myxococcales (OTU7571)	neg
	Gemmatimonadetes (OTU389)	neg
2	Bacteroidetes, *Flavobacterium succinicans* (OTU112)	pos
(Weeds, 1.016)	Proteobacteria, Gammaproteobacteria (OTU91)	pos
	Proteobacteria, Betaproteobacteria (OTU20)	pos
	Proteobacteria, Betaproteobacteria (OTU383)	pos
	Bacteroidetes, *Sphingobacterium multivorum* (OTU361)	pos
2	Archaea, *Candidatus Nitrososphaera SCA1145* (OTU13579)	neg
	Archaea, *Candidatus Nitrososphaera* (OTU77)	neg
	Acidobacteria, Koribacteraceae (OTU168)	neg
	Archaea, *Candidatus Nitrososphaera SCA1145* (OTU6)	neg
	Cyanobacteria, *Phormidium* (OTU173)	neg
3	Archaea, *Candidatus Nitrososphaera SCA1145* (OTU13579)	pos
	Archaea, *Candidatus Nitrososphaera* (OTU77)	pos
	Proteobacteria, Betaproteobacteria (OTU96)	pos
	Actinobacteria, *Nocardioides* (OTU74)	pos
	Actinobacteria, Gaiellaceae (OTU1805)	pos
3	Archaea, *Candidatus Nitrososphaera* (OTU116)	neg
(Oat, -1.032)	Archaea, *Candidatus Nitrososphaera* (OTU151)	neg
	Acidobacteria (OTU78)	neg
	Actinobacteria (OTU32)	neg
	Archaea, *Candidatus Nitrososphaera* (OTU134)	neg

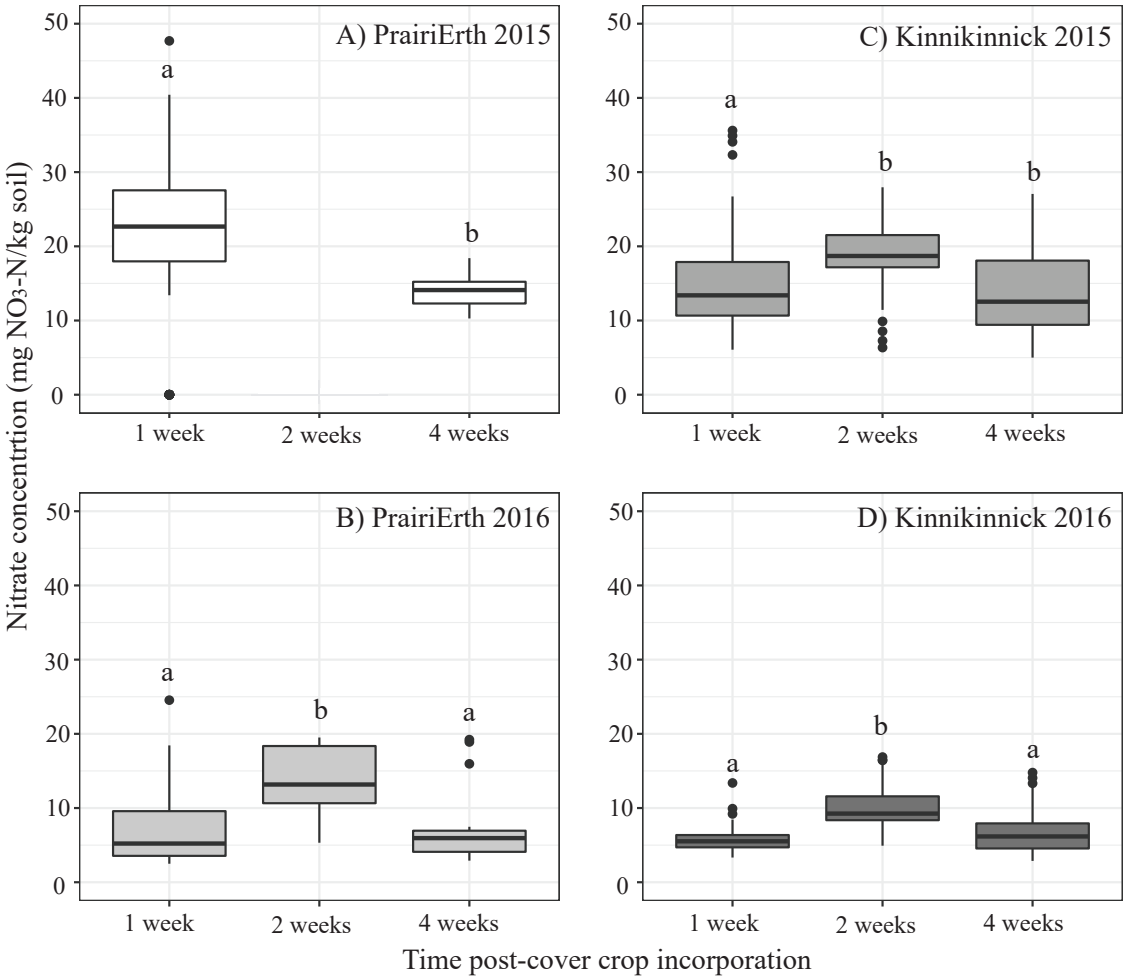
Table 7. Top loading fungal OTUs for the PLSR model at Kinnikinnick. Components correspond to the components listed in Table 4. Loadings are either the top positive ("pos") or negative ("neg") values.

Component	Fungal OTU	Loading
1	Zygomycota, *Mortierella* (OTU48)	pos
	Ascomycota, Helotiales (OTU98)	pos
	Ascomycota (OTU65)	pos
	Ascomycota, Lasiosphaeriaceae (OTU65)	pos
	Ascomycota, Phaeosphaeriaceae (OTU94)	pos
1	Zygomycota, *Mortierella* (OTU13)	neg
(Weeds, -0.872	Basidiomycota, Psathyrellaceae sp (OTU27)	neg
Mustard, -0.495)	Zygomycota, *Mortierella exigua* (OTU16)	neg
	Zygomycota, *Mortierella* (OTU3981)	neg
	Ascomycota, Xylariales (OTU128)	neg
2	Ascomycota (OTU19)	pos
(Weeds, 0.537)	Ascomycota, Dothideomycetes (OTU40)	pos
	Ascomycota, *Pyrenochaetopsis leptospora* (OTU121)	pos
	Ascomycota, Lasiosphaeriaceae (OTU61)	pos
	Ascomycota, *Aspergillus fischeri* (OTU46)	pos
2	Ascomycota, Lasiosphaeriaceae (OTU18)	neg
(Mustard, -0.853)	Zygomycota, *Rhizopus arrhizus* (OTU33)	neg
	Zygomycota, *Mortierella exigua* (OTU16)	neg
	Ascomycota, *Clonostachys rosea* (OTU55)	neg
	Basidiomycota, Agaricales (OTU193)	neg
3	Zygomycota, *Mortierella* (OTU48)	pos
	Ascomycota, Chaetomiaceae (OTU97)	pos
	Ascomycota (OTU19)	pos
	Ascomycota, *Alternaria eichhorniae* (OTU8)	pos
	Ascomycota (OTU58)	pos
3	Ascomycota (OTU21)	neg
(Oat, -1.005)	Zygomycota, *Mortierella* (OTU13)	neg
	Zygomycota, *Rhizopus arrhizus* (OTU33)	neg
	Ascomycota, *Trichocladium asperum* (FUN3930)	neg
	Basidiomycota, *Coprinellus marculentus* (OTU155)	neg

Table 8. Top loading fungal OTUs for the PLSR model at PrariErth. Components correspond to the components listed in Table 4. Loadings are either the top positive ("pos") or negative ("neg") values.

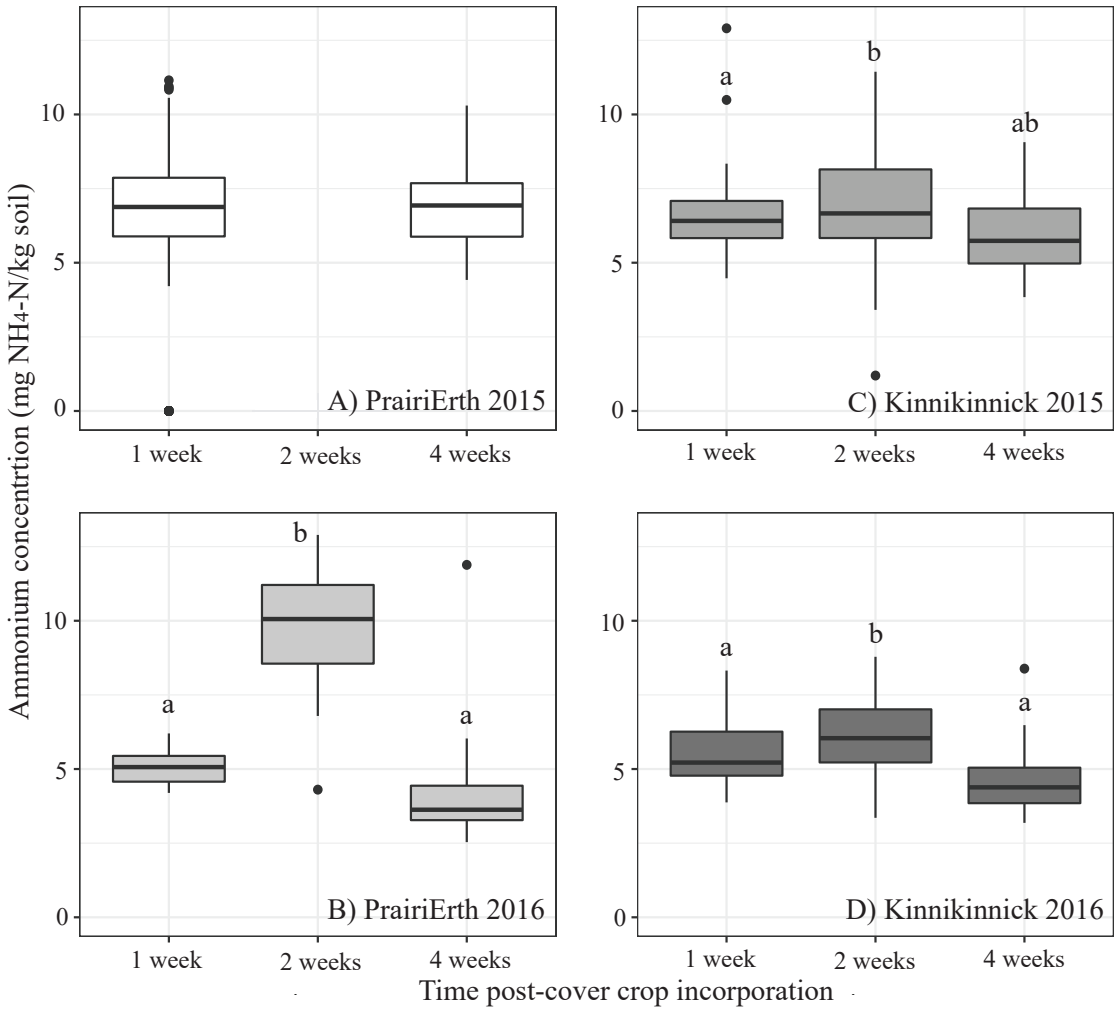
Component	Fungal OTU	Loading
1	Ascomycota, Pyronemataceae (OTU170)	pos
(Mustard, 1.002)	Ascomycota, Lasiosphaeriaceae (OTU4)	pos
	Ascomycota (OTU86)	pos
	Ascomycota, *Trichocladium asperum* (OTU3)	pos
	Ascomycota, *Chalara* (OTU4176)	pos
1	Ascomycota, *Monographella cucumerina* (OTU5)	neg
	Ascomycota, Nectriaceae (OTU1)	neg
	Ascomycota, *Colletotrichum anthrisci* (OTU71)	neg
	Ascomycota, *Monographella cucumerina* (OTU2343)	neg
	Ascomycota, Lasiosphaeriaceae (OTU18)	neg
2	Ascomycota, Lasiosphaeriaceae (OTU4)	pos
(Weeds, 1.054)	Basidiomycota, Ceratobasidiaceae (OTU44)	pos
	Ascomycota, Chaetomiaceae (OTU15)	pos
	Ascomycota, *Monographella cucumerina* (OTU5)	pos
	Ascomycota, *Fusarium* (OTU11)	pos
2	Ascomycota, *Penicillium* (OTU38)	neg
(Mustard, -0.141)	Fungi (OTU113)	neg
	Basidiomycota, *Guehomyces pullulans* (OTU25)	neg
	Ascomycota, *Pochonia boninensis* (OTU3908)	neg
	Ascomycota, *Phoma* (OTU10)	neg
3	Ascomycota, *Penicillium* (OTU38)	pos
(Weeds, 0.230)	Ascomycota, *Acremonium persicinum* (OTU107)	pos
	Zygomycota, *Mortierella* (OTU13)	pos
	Ascomycota, *Fusarium oxysporum* (OTU7)	pos
	Ascomycota (OTU173)	pos
3	Ascomycota, Lasiosphaeriaceae (OTU4)	neg
(Oat, -1.030)	Ascomycota, Myrothecium verrucaria (OTU176)	neg
	Ascomycota, Chaetomiaceae (OTU15)	neg
	Basidiomycota, *Guehomyces pullulans* (OTU25)	neg
	Fungi (OTU54)	neg

Figure 1



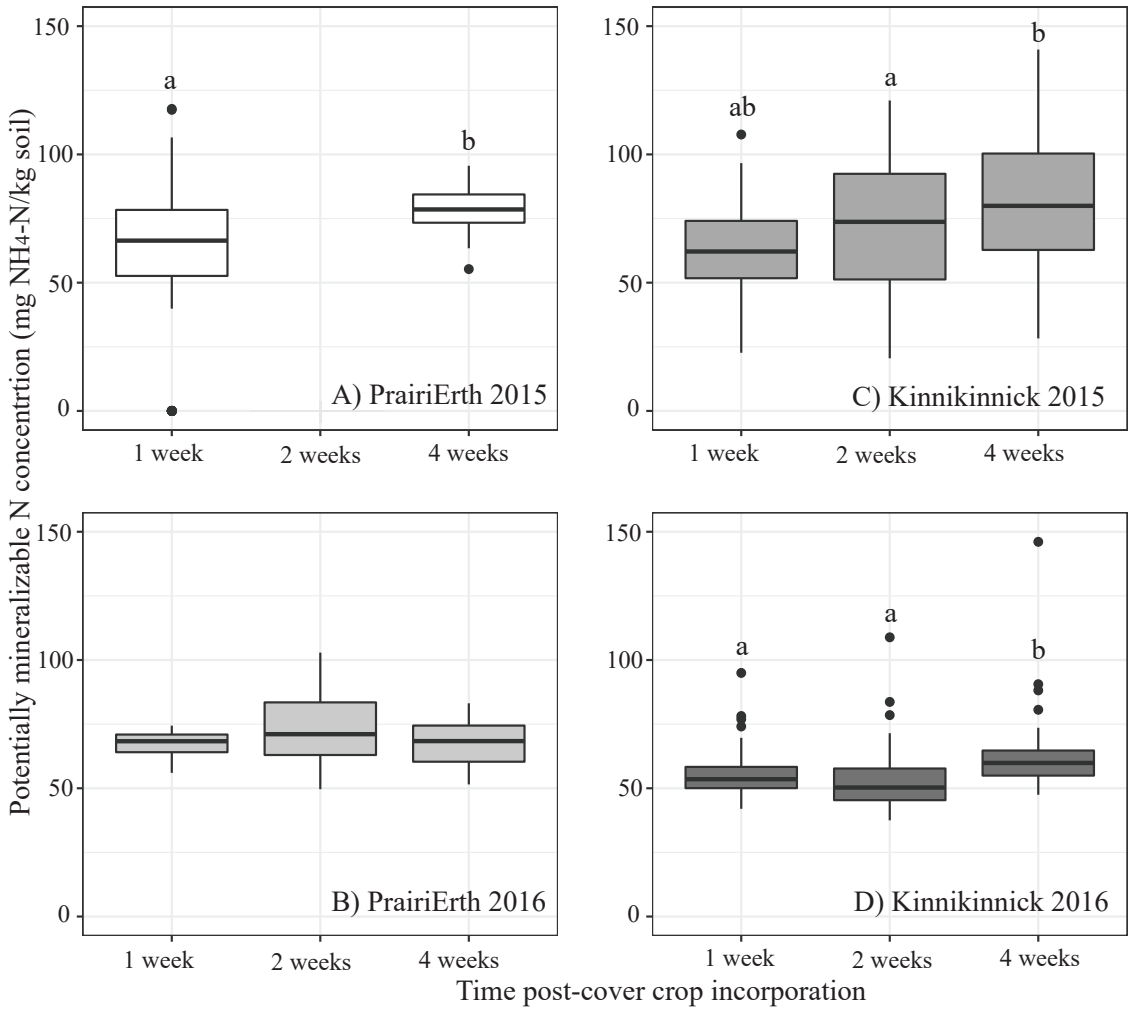
Soil nitrate concentrations over the sampling period at A) PrariErth farm in 2105, B) PrairiErth farm in 2016, C) Kinnikinnick farm in 2015 and D) Kinnikinnick farm in 2016. No samples were collected at the 2-week post-incorporation sample date at PrairiErth in 2015. Letters indicate significant differences (Table 1, Tukey HSD post-hoc test, $p < 0.05$).

Figure 2



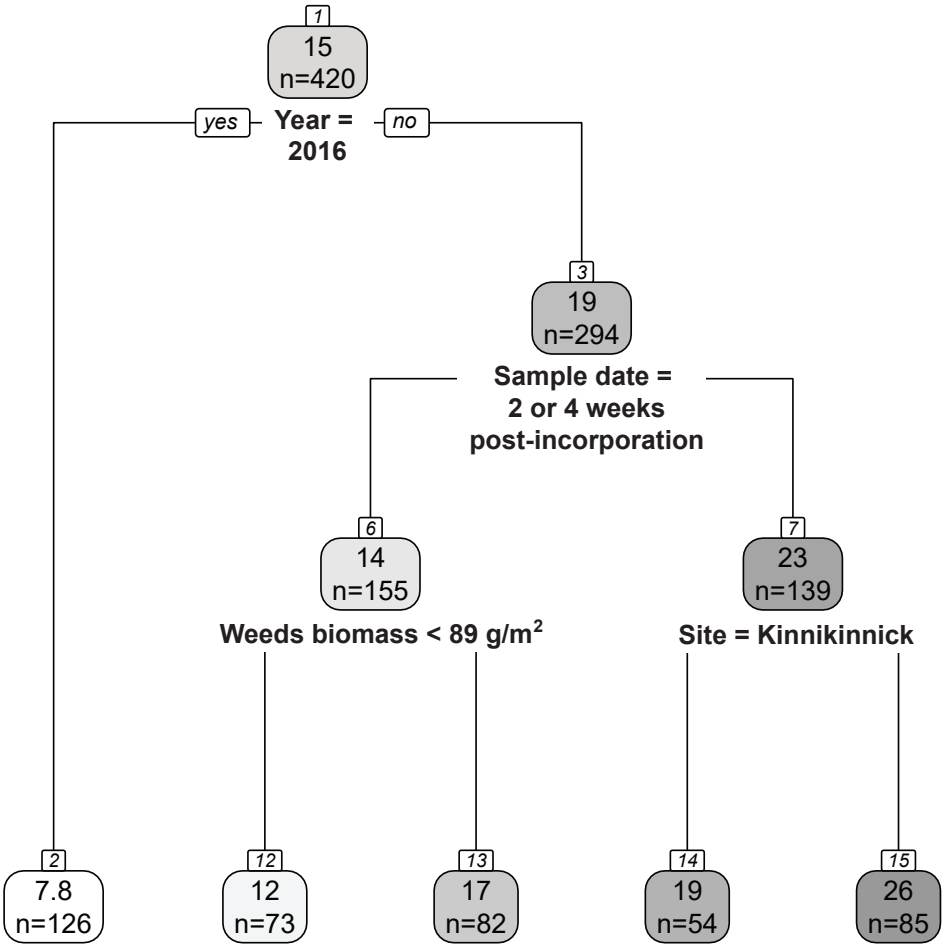
Soil ammonium concentrations over the sampling period at A) PrariErth farm in 2105, B) PrairiErth farm in 2016, C) Kinnikinnick farm in 2015 and D) Kinnikinnick farm in 2016. No samples were collected at the 2-week post-incorporation sample date at PrairiErth in 2015. Letters indicate significant differences (Table 1, Tukey HSD post-hoc test, $p < 0.05$).

Figure 3



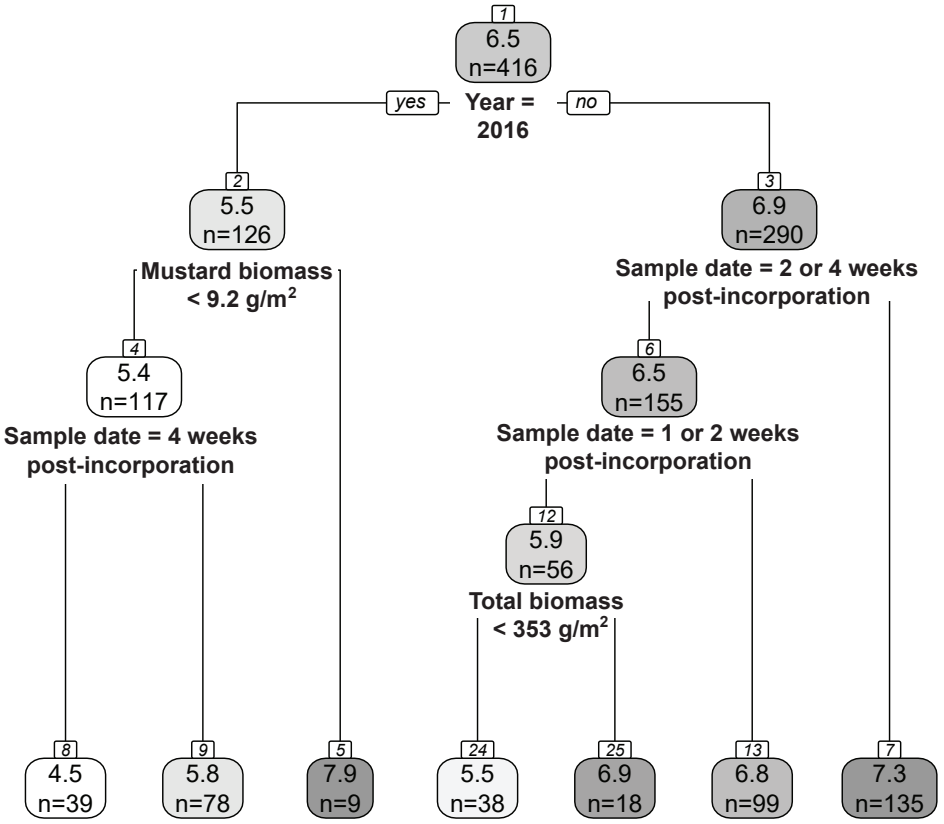
Soil potentially mineralizable nitrogen concentrations over the sampling period at A) PrariErth farm in 2105, B) PrairiErth farm in 2016, C) Kinnikinnick farm in 2015 and D) Kinnikinnick farm in 2016. No samples were collected at the 2-week post-incorporation sample date at PrairiErth in 2015. Letters indicate significant differences (Table 1, Tukey HSD post-hoc test, $p < 0.05$).

Figure 4



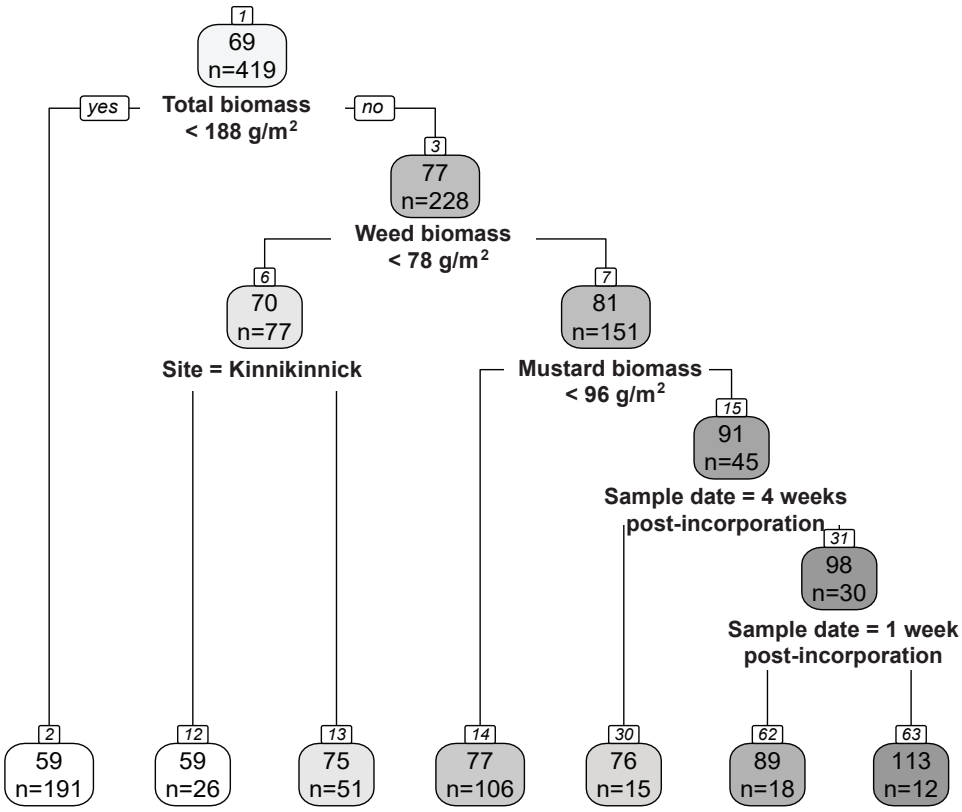
Regression tree of soil nitrate concentration with the predictors of cover crop biomass, sample period, site and year. Reported values are in mg NO_3-N/kg soil, and n = number of samples included in the pool. A darker grey color indicates a high nitrate concentration. The tree was pruned to cp = 0.015 with a cross-validation error of 0.38.

Figure 5



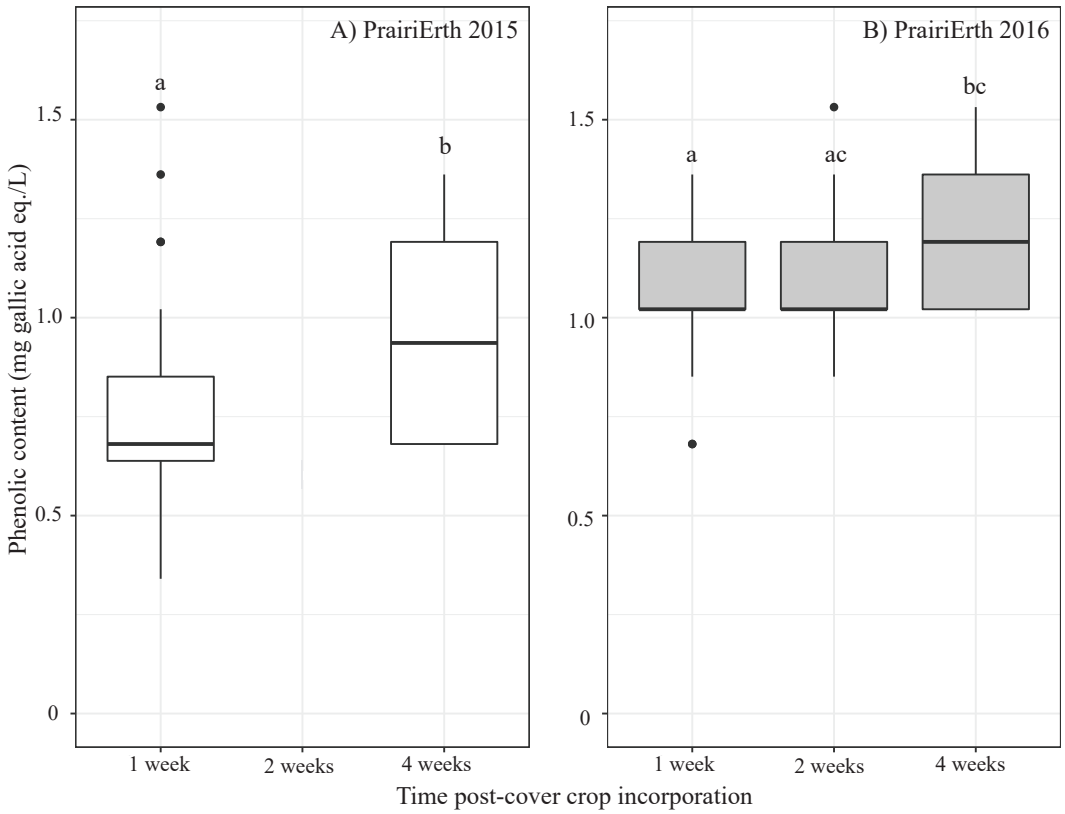
Regression tree of soil ammonium concentration with the predictors of cover crop biomass, sample period, site and year. Reported values are in mg NH_4-N/kg soil, and n = number of samples included in the pool. A darker grey color indicates a high nitrate concentration. The tree was pruned to cp = 0.013 with a cross-validation error of 0.85.

Figure 6



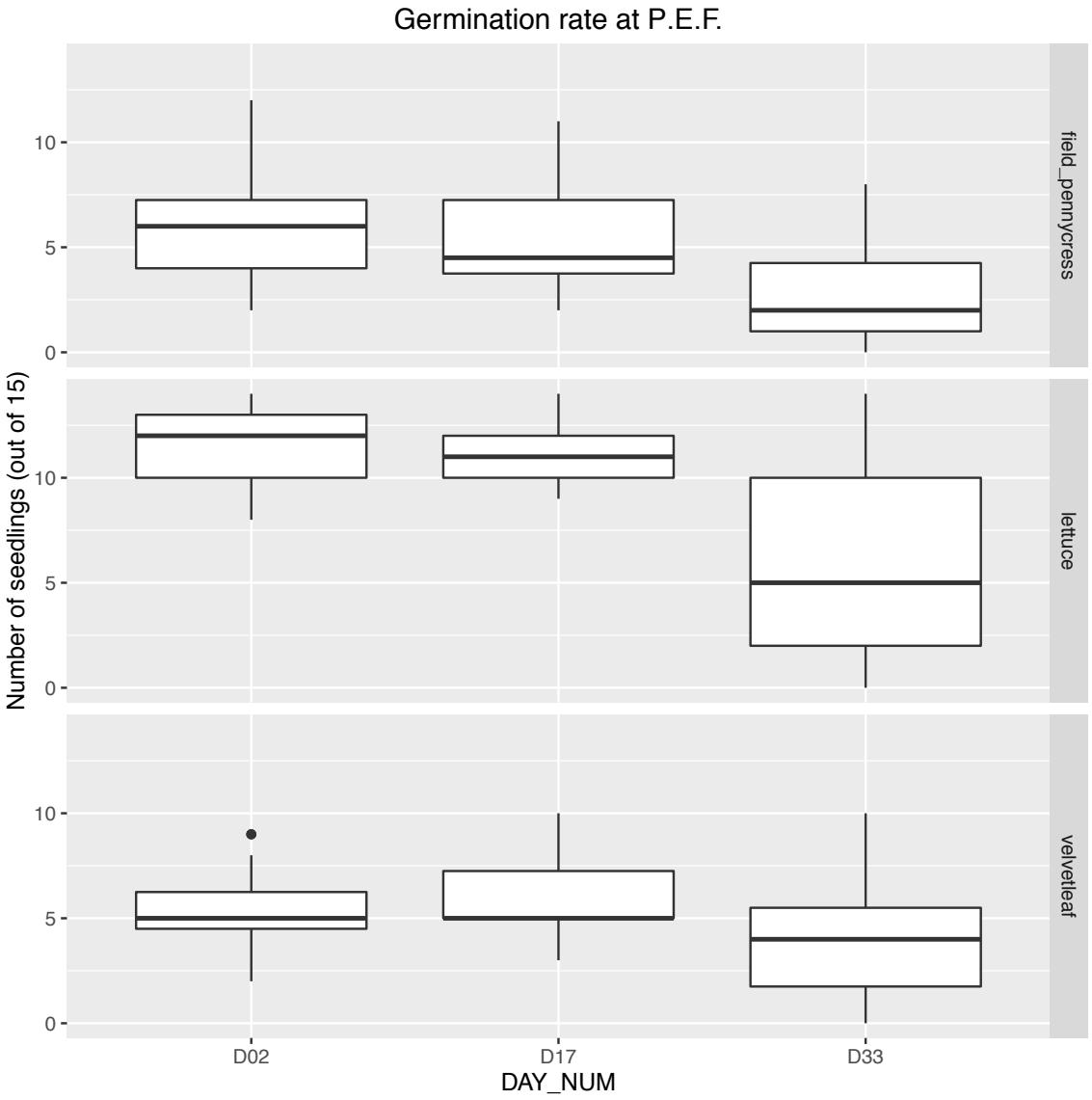
Regression tree of soil PMN concentration with the predictors of cover crop biomass, sample period, site and year. Reported values are in mg NH_4-N/kg soil, and n = number of samples included in the pool. A darker grey color indicates a high nitrate concentration. The tree was pruned to cp = 0.023 with a cross-validation error of 0.76.

Figure 7



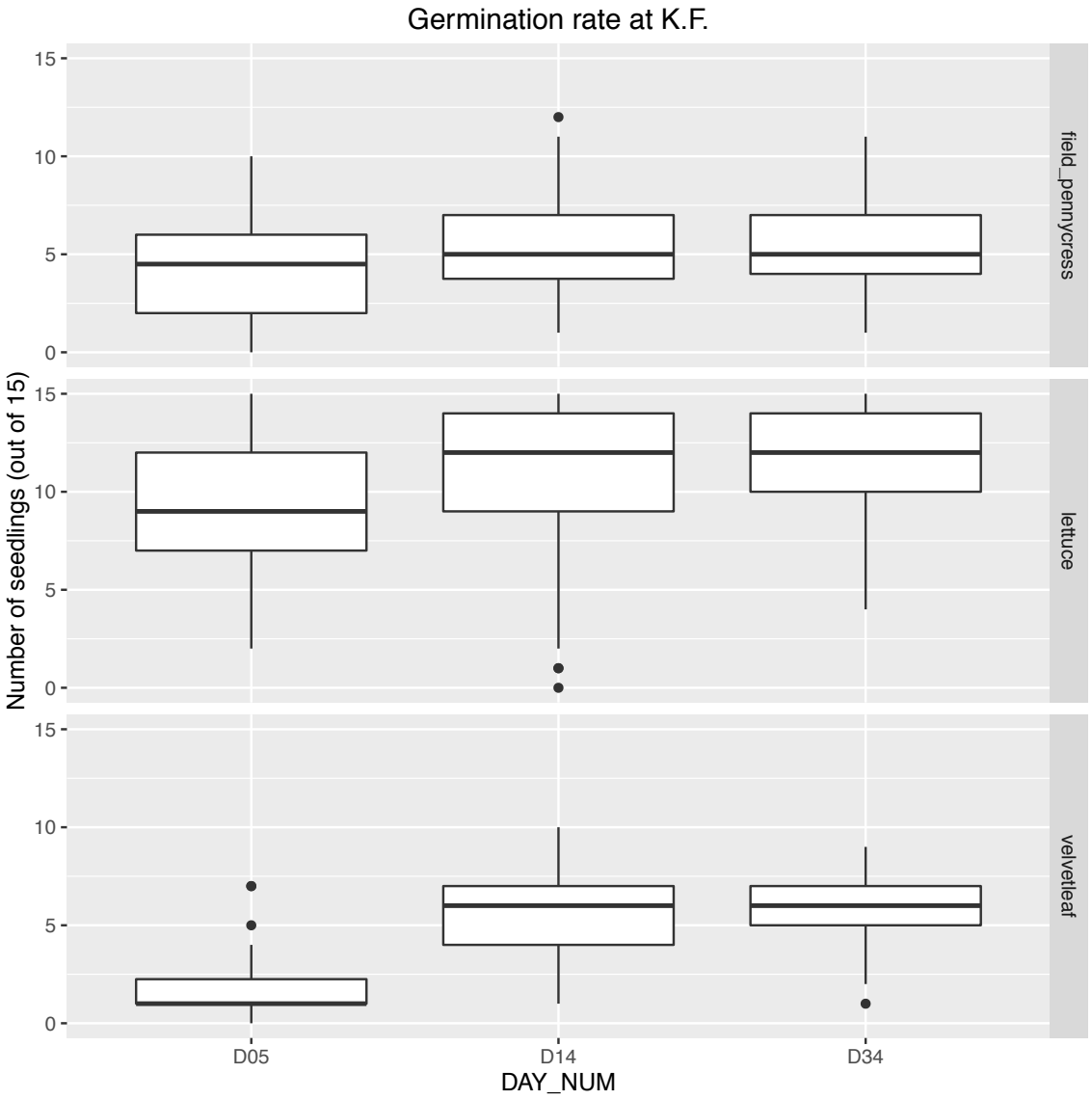
Soil phenolic concentrations over the sampling period at A) PrariErth farm in 2105 and B) PrairiErth farm in 2016. No samples were collected at the 2-week post-incorporation sample date at PrairiErth in 2015. Letters indicate significant differences (ANOVA, Tukey HSD post-hoc test, $p < 0.05$.

Figure 8



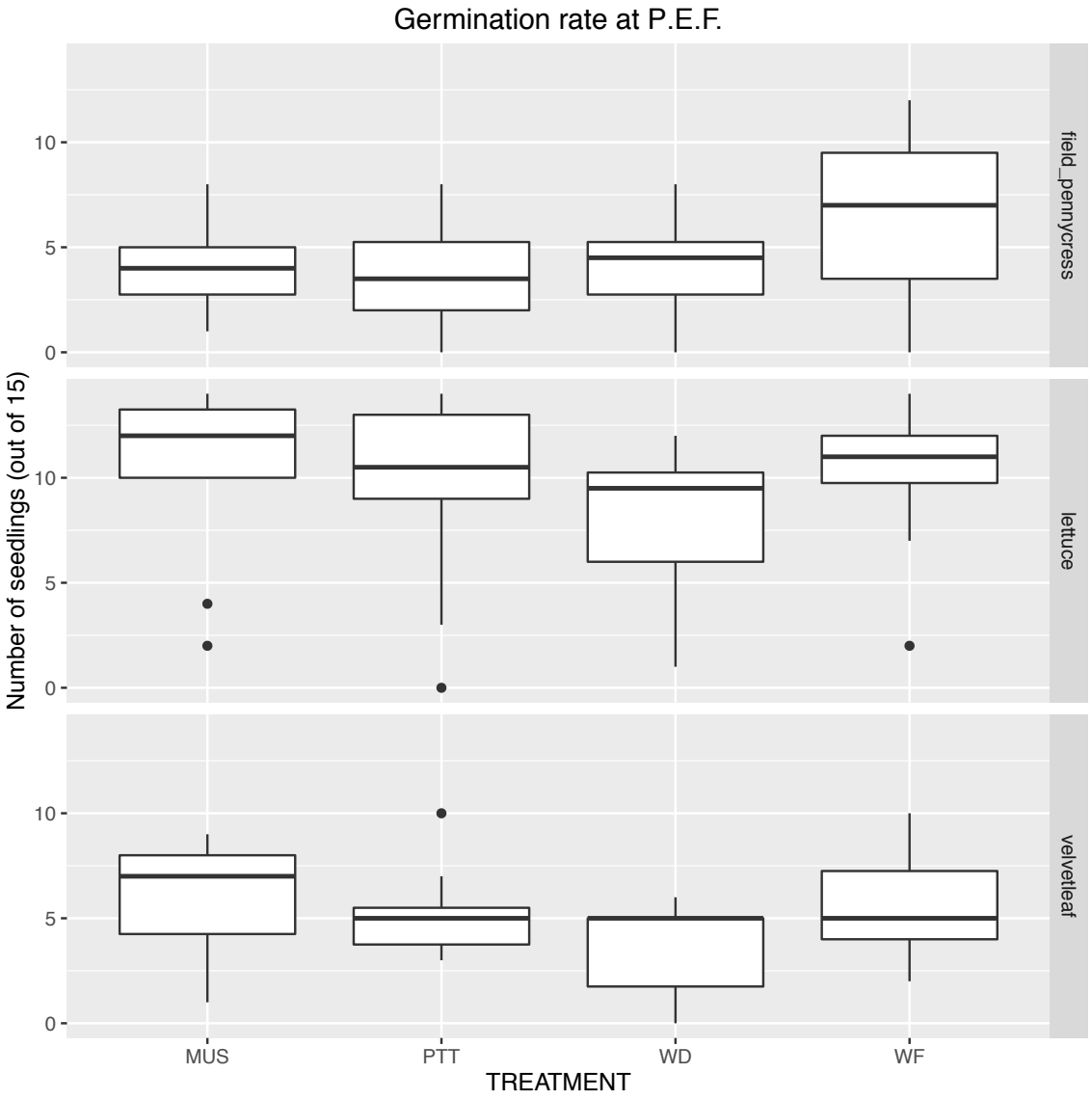
Germination rate by cover crop treatment of two weeds and one crop at one of our working farms in 2016. Box plots summarize the number of germinating seeds across all treatment plots at each time point (day 2, 17, and 33 post-termination).

Figure 9



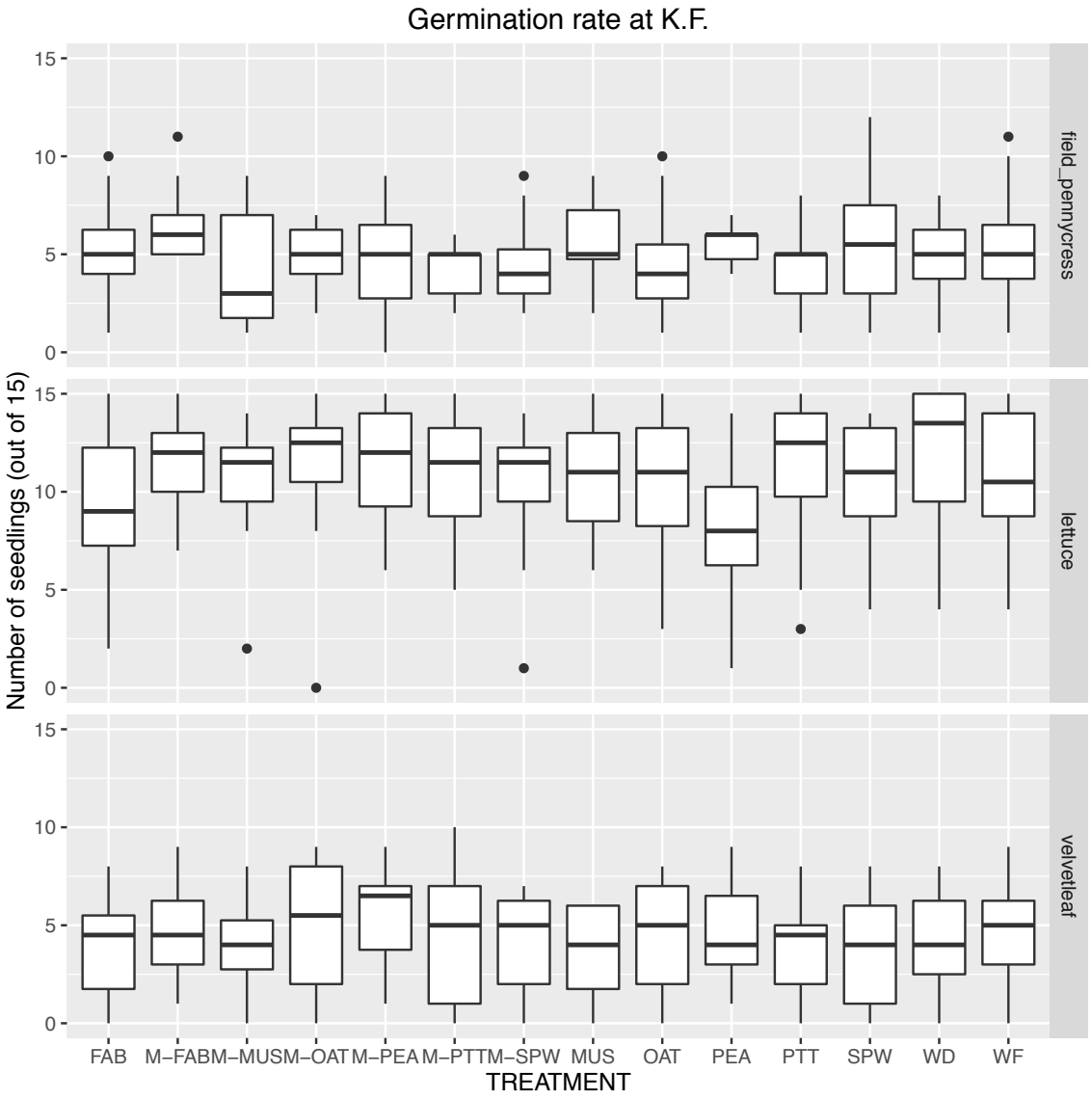
Germination rate by cover crop treatment of two weeds and one crop at one of our working farms in 2016. Box plots summarize the number of germinating seeds across all treatment plots at each time point (day 5, 14, and 34 post-termination).

Figure 10



Germination rate by cover crop treatment of two weeds and one crop at one of our working farms in 2016. Box plots summarize the number of germinating seeds from each set of treatment plots (4 replicates) over all time points (day 2, 17, and 33 post-termination). Codes for treatments are as follows: MUS = goliath mustard; PTT = purple-top turnips; WD = no cover control (including a natural weed community); WF = weed-free controls (no plants).

Figure 11



Germination rate by cover crop treatment of two weeds and one crop at one of our working farms in 2016. Box plots summarize the number of germinating seeds from each set of treatment plots (4 replicates) over all time points (day 5, 14, and 34 post-termination). Codes for treatments are as follows: FAB = fava bean; MUS = goliath mustard; OAT = oats; PEA = field pea; PTT = purple-top turnips; SPW = spring wheat; WD = no cover control (including a natural weed community); WF = weed-free controls (no plants); treatments prepended with M- are multi-species combinations that include all species except for the one indicated (e.g. M-FAB includes all species except for fava beans).