Improving Rotational Partners: intraspecies variation for pea cover cropping traits

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Research Article

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Abstract

Purpose: To improve cover crops as rotational partners, intraspecies variation for cover cropping traits such as nutrient mobilization, carbon deposition, and beneficial microbial recruitment must be identified. The majority of research on cover crops has focused on interspecies comparisons for cover cropping variation with minimal research investigating intraspecies variation.

Methods: To address if variation of cover cropping traits is present within a cover cropping species, we grew 15 diverse accessions (four modern cultivars, three landraces, eight wild accessions) of pea in an organic setting. We measured various cover cropping traits such as nutrient mobilization, soil organic matter deposition, microbial recruitment, and quantified the effect of pea accession on the growth and yield of a subsequently planted corn crop.

Results: We found that domestication history and genotype of pea had a significant effect on soil properties: C%, N%, manganese, magnesium, sodium, calcium, and effective CEC, and the yield of the subsequent corn crop. Additionally, no variation for prokaryotic recruitment (alpha and beta diversity) was observed within pea, however we did observe significant variation for fungal recruitment (alpha and beta diversity) due to domestication and accession. In conclusion, our results revealed the presence of intraspecies variation for cover cropping traits which, may have impacted the rotational values of pea accessions.

Conclusions: Our results demonstrate that cover crops can be improved as rotational partners to ultimately boost crop yields in sustainable agroecosystems.

Introduction

Cover crops are widely utilized in agricultural systems due to their beneficial impacts on crop yields, above and belowground biodiversity, disease and weed suppression, and overall soil health (reviewed in (Hartwig & Ammon, 2002; Miguez & Bollero, 2005; Sharma et al., 2018; Snapp et al., 2005). Due to these benefits, the number of agricultural acres being cover cropped in the United States has increased by 49.7% from 2012 to 2017 (National Agricultural Statistics Service, 2019). Despite the growing popularity of cover crops and their critical role in sustainable agriculture, minimal effort has gone into improving cover crops as rotational partners. The majority of cover crop research has been focused on comparing cover cropping traits between species or species mixtures, whereas few studies have investigated differences between cover cropping traits within species. Identifying intraspecies differences may provide the foundation for improving the rotational value of cover crops, which we define as a measure of how well a cover crop increases the yield of a subsequent crop (Marques et al., 2020). To this end, increasing rotational value may potentially help offset the estimated 19.2% yield gap between conventional and sustainable agricultural practices (Ponisio et al., 2015; Sharma et al., 2018). Reducing the yield gap between these systems through rotational value improvement is critical for making sustainable agriculture practices a feasible solution to feed the ever-growing human population (Barbieri et al., 2019; Licker et al., 2010; Ponisio et al., 2015; Sharma et al., 2018).

Differences in cover cropping traits among species and families including weed and disease suppression (Snapp et al., 2005), soil organic matter deposition (Johanning, 2014), nutrient mobilization (Hallama et al., 2019), and below (Liang et al., 2014; Wagg et al., 2011) and aboveground (Finney & Kaye, 2017) biodiversity improvement, have been well documented in various agroecosystems (reviewed in Hartwig & Ammon, 2002; Sharma et al., 2018). For instance, when compared to cereal cover crops, legume cover crops are not well suited for weed suppression (Chauhan et al., 2012; Hodgdon et al., 2016) but are more efficient at increasing soil carbon and nitrogen (Snapp et al., 2005). Despite these well-established cover crop generalizations, most cover cropping studies are limited in that they use a single variety or accession to represent an entire cover cropping species. The use of a single accession or variety is problematic because within-species variation for agronomically important traits, such as abiotic tolerance (reviewed in Bita & Gerats, 2013; Bosetti et al., 2012; Coyne et al., 2020; Marques et al., 2021; Rao et al., 2016; Sivasakthi et al., 2019; Von Wettberg et al., 2018), and resistance to diseases (Ahmad et al., 2010; Vasudevan et al., 2014) and pests (Broekgaarden et al., 2011; Rakha et al., 2017; Von Wettberg et al., 2018), have been consistently found across crops. As a result, intraspecies variation for cover cropping traits such as nutrient mobilization, organic matter deposition, and beneficial soil microbial recruitment most likely exist. Therefore, cover cropping results from a single variety or accession must be carefully extrapolated since it may lead to incorrect generalizations about crop families or species.

In addition to increasing the number of accessions and varieties used in studies, crop wild relatives (CWRs) should be incorporated in cover cropping research. Tribouillois et al. (2015) suggested that domestication has reduced adaptive strategies and modified leaf trait syndromes in cover crops. Thus, the impacts of genetic bottlenecks associated with domestication and modern breeding (Doebley et al., 2006) may also be affecting the genetic and phenotypic diversity of cover crops. To alleviate restrictions on genotypic and phenotypic

diversity, incorporating genetic material from CWRs that have not undergone domestication may help increase intraspecies variation in cover cropping studies.

Here we test if rotational traits and values vary within cover cropping species by utilizing a modified plant-soil feedback (PSF) framework (Marques et al., 2020) and an assortment of pea accessions with varying domestication histories (modern cultivars, landraces, and wild relatives). We measured various cover cropping traits such as nutrient mobilization, organic matter deposition, microbial recruitment, and rotational values. We hypothesized that cover cropping traits would vary between pea accessions and domestication histories, as other agronomically important traits have been seen to vary between pea accessions (e.g., Coyne et al., 2020). The presence of variation in cover cropping traits would suggest that rotational values in cover crops can be enhanced to increase yields in agroecosystems.

Methods

Plant Material

Fifteen pea accessions were used in this experiment. All accessions were requested from the USDA-NPGS and then amplified in Burlington, Vermont. Eight of the accessions, W6 26154, W6 26154 PSP, W6 26157, W6 26157 PSP, W6 26159, W6 26160 PSP, W6 26161, and W6 26161 PSP, were wild accessions from the country of Georgia. The remaining accessions, PI 269761, PI 269761 PSP, PI 639977 PSP, and PI 639981 PSP, were modern cultivars originating from the Czech Republic (2) and Bulgaria (2) respectively, and PI 577142, W6 3674, and W6 3675 were landraces from Nepal (Table 1).

Experimental Design

Four replicates of each pea accession and one control (no cover crop) were grown in a randomized block design in an organic field at the University of Vermont Horticulture Research Center in South Burlington, Vermont. Approximately 20.41 g of each pea accession was planted at a depth of ~ 2.54 cm in 2.8m² plots. The sowing rate of ~ 7.3 g/m² and the 2.54 cm planting depth mimicked the recommended cover cropping plant density of 65 lbs/ac for cover cropping peas (Berg et al., 2017; USDA, 2019). Before planting, all seeds were sterilized with a 1% bleach solution to ensure no microbes were introduced to the plot via the seed coat. Plots were irrigated as needed. Pea plants were grown for 44 days, after which soil rhizosphere samples were collected, and the total number of plants in the plot, the average plant height, and the average aboveground biomass were recorded. To calculate the average plant height, three of the most center plants in the plot were selected, and plant height (base of the plant at soil level to the top of the stem) was recorded and averaged. The plants were uprooted after their height was measured, and soil rhizosphere samples were collected. The rhizosphere was defined as any soil still clinging to the plant's root after the plant was uprooted. For control plots, bulk soil was taken at an approximate depth of 15 cm at the center of the plot. To calculate the average aboveground biomass, the three uprooted plants' aboveground portions were separated from their belowground portions and oven-dried for 48 hours at 49°C and then weighed using an analytical scale. After pea plant measurements were recorded, soil core samples were collected at the center of each plot at an approximate depth of 15 cm using a 7.5 cm diameter soil recovery AMS auger. Soil samples and the previously listed plant measurements were obtained from the plots' centers to avoid edge and interacting effects from neighboring plots. Soil core samples were then sent to the University of Vermont Agricultural and Environmental Testing Laboratory, where they were tested for pH, % nitrogen (N), % carbon (C), % soil organic matter, phosphorus, potassium, aluminum, calcium, iron, magnesium, manganese, sulfur, zinc, and effective cation exchange capacity (CEC). After the soil core samples were obtained, the remaining plots were hand-harvested by cutting the plant's stem at the soil level; this was done to minimize soil disturbance in the plot.

After harvesting pea plants, the sweet corn organic variety "Enchanted" was hand planted in the plots according to the manufacturer's specifications. "Enchanted" was used because it is a neonicotinoid-free and late-season maturing variety that reaches maturity 78 days after sowing. Eighty days after sowing, the number of corn plants, average plant height, average aboveground biomass (cob and vegetative), and relative chlorophyll content were recorded for each plot (explained in more detail below). The same protocol used to calculate the average plant height and aboveground biomass of the pea plants was used for the corn plants. If a cob showed signs of pest damage, the measurement for that plant was excluded, and another plant in the plot was measured. For chlorophyll measurements, the youngest fully developed leaf was measured for leaf chlorophyll content using a Leaf Photosynthesis MultispeQ V1.0 (East Lansing, MI). Only plants closest to the direct center of the plot were sampled to avoid edge and interacting effects from neighboring plots.

Microbiome Measurements

Microbial DNA was extracted from bulk soil control plots and pea rhizosphere samples using QIAGEN DNeasy PowerSoil Kits. Before DNA extraction, all samples were treated with propidium monoazide utilizing the manufacturer's protocol (Biotium) to prevent the extraction and amplification of soil relic DNA, which can potentially skew microbial diversity estimates (Carini et al., 2016). After extraction, DNA samples were sent to LC Sciences (Houston, TX) for DNA library preparation, and sequenced for prokaryotic 16S rRNA (V3 and V4 regions) and internal transcribed spacer (ITS2) genes using a Illumina MiSeq sequencer. Only one rhizosphere sample from each plot was sequenced. Sequence data was then processed for amplicon sequence variants (ASVs) using the requisite quality assurances in the Qiime2 and Dada2 pipelines (Callahan et al., 2016). The taxonomy of the ASVs was characterized using the Ribosomal Database Project (RDP version 11.3), NCBI 16S Microbial Database, and the Greengenes databases.

Statistical Analysis

To calculate the effect of pea accessions on soil chemistry and the growth of the subsequently planted corn, a modified PSF framework was used, and the magnitude and direction of PSF in each accession were calculated for all measurements (Ingerslew & Kaplan, 2018; Mariotte et al., 2018; Marques et al., 2020). For all measurements, the following formula was used:

$$PSF_s = \ln\left(\frac{SM_s}{SM_c}\right)$$

Where SM_s is the recorded soil measurement or corn measurement of the plot, and SM_c is the average soil or corn measurement for all control plots. Additionally, the same metric was used to calculate the rotational value (RV), where SM_s is the average corn cob weight of the plot, and SM_c is the average corn measurement of all control plots. The use of a standardized PSF and RV provides a clear understanding of pea accessions' effect on soil chemistry and the subsequently planted crop. If PSF_s or RV were less than 0, soil or corn measurements were lower than control measurements. If PSF_s or RV were greater than 0, soil or corn measurements were higher than control measurements. Lastly, if PSF or RV was equal to 0, then soil or corn measurements were similar to control measurements.

A generalized linear mixed model was used to test for significant differences among accessions and histories (modern cultivar, landrace, wild) effects on soil chemistry and corn growth and yield (Bates et al., 2014). For soil measurement GLM models, block was used as a random variable, and the total aboveground biomass of the plot was used as a covariate. The total aboveground biomass of the plot was calculated by multiplying the number of pea plants in the plot by the average pea aboveground biomass of the plot. Although not a precise measure, this proxy gave an approximate estimate of the total aboveground biomass of the plot. This covariate was used to account for differences in pea plant size between accessions. For corn measurement GLM models, block was again used as a random variable, and the number of corn plants in the plot was used as a covariate. This covariate was used to account for differences in the number of corn plants present in each plot. A Tukey's HSD post-hoc test was used to test for significant differences between accessions and history groups. The effects of accession and history (domesticated or wild) on soil and corn measurements were analyzed separately, as we wanted to test for significant differences between accessions. If both factors were included in a single model, accession would become nested within history and be categorized as a random term, thus preventing the identification of significant differences between accessions.

To test for linear correlation between rotational value and PSF soil calculations and pea measurements, the Pearson correlation coefficient was calculated for rotational values versus pea measurements or PSF soil calculations. Additionally, to test for linear correlation between pea aboveground biomass of the plot and soil measurements, the Pearson correlation coefficient was calculated for all PSF soil calculations versus pea aboveground biomass. All statistical analyses were performed in R (www.r-project.org).

Microbial Analysis

Amplicon sequence variants (ASVs) were rarefied to 90% of the minimum sample depth in the dataset. Rarefied ASVs were used to calculate alpha diversity for both history and accessions using Chao1, Shannon, Simpson, abundance-based coverage estimators, and Fischer indices. Alpha diversity was calculated using the "Phyloseq" and the "microbiomeSeq" R packages. A one-way (accession or history) ANOVA and a Tukey's HSD posthoc test were used to determine if alpha microbial diversity was significantly different between accessions and history groups. Additionally, the Bray-Curtis dissimilarity method with a Hellinger transformation was used to calculate beta diversity for accession and history. The dissimilarity matrices were then analyzed with distance based redundancy analysis (dbRDA)

and permutational multivariate analysis of variance (PERMANOVA). All beta diversity analysis was conducted using the "Vegan" package and RDA graphs were made using the “ampvis2” package in R. Furthermore, using the "Vegan" package in R, a redundancy analysis was performed to calculate the amount of variation present in species explained by accession history, respectively. To test for differencial abundance of ASVs for accessions, the “differentialTest” function (controlling the effect of domestication history on dispersion) from "Corncob" package in R was used (Martin et al., 2020). Lastly, to test for linear correlation between rotational values and microbial presence at the phylum level, the Pearson correlation coefficient was calculated for rotational values versus all normalized microbial groups using R's "psych" package. Microbial and fungal communities were analyzed separately.

Results

PSF Values of Soil Measurements

The Plant-soil feedback (PSF) values of soil chemistry measurements varied between modern cultivars, landraces, and wild peas, with modern cultivars and landraces generally having positive or neutral values and wild peas having negative or neutral values (Figure 1). Significant differences in PSF values for %N (P = .016), %C (P = .046), and manganese (P = .044) were observed between modern cultivars, landraces and wild peas, with domesticated (modern cultivar, landraces) peas having higher PSF values than wild peas (Figure 1). Conversely, for potassium (P = .110), the PSF value of wild peas was higher than modern cultivars. For all other measurements pH (P = .195), magnesium (P = .086), iron (P = .073), phosphorous (P = .099), organic matter (P = .095), calcium (P = .515), sulfur (P = .162), zinc (P = .433), sodium (P = .174), aluminum (P = .722), and CEC (P = .419) were non-significant between domestication history.

Similar to domestication history, accession variation of soil PSF values were widespread, with accessions having positive, negative, or neutral values (Figure 2). Accessions varied significantly in PSF values for calcium (P = .013), magnesium (P = .002), manganese (P = .016), sodium (P = .007), CEC (P = 0.012), and % C (P = < .001) (Figure 2). While accessions did not significantly differ in PSF values for %N (P = .060), pH (P = .265), organic matter (P = .304), phosphorus (P = .203), potassium (P = .069), aluminum (P = .089), iron (P = .371), sulfur (P = .094), and zinc (P = .078).

Additionally, the aboveground biomass of wild and domesticated plants significantly affected soil PSF values for pH (P = .010), potassium (P = .035), and magnesium (P = .007). However, only A significant negative correlation between pH and total aboveground biomass (r = -.263, P = 0.042) and a nearly significant negative correlation between magnesium and total aboveground biomass (r = -.243, P = .062) were observed.

Recruited Rhizosphere Communities

Prokaryotic Communities

α-diversity indices Chao1 (P = 0.433, P = 0.805), Shannon (P = 0.213, P = 0.638), Simpson (P = .311, P = .117), abundance-based coverage estimators (P = 0.487, P = 0.825) and Fisher (P = 0.383, P = 0.805) were non-significant for prokaryotic rhizosphere communities for both domestication history and accession, respectively (Supplemental Figure S1). Additionally, β-diversity (Bray-Curtis dissimilarity) between accessions (PERMANOVA, P = 0.958) was not significantly different. However, β-diversity for domestication history (PERMANOVA, P = 0.059) was significant at α = .075. Furthermore, db-RDA revealed that domestication history accounts for 78.9% (RD1 55.7%, RD2 23.2%) of the variation found in the prokaryotic microbiome (Figure 3A).

Lastly, significant differences between accessions for differential abundance were observed for ten ASV, unclassified Gemmatimonadetes (ASV 20, P = 0.03), unclassified Microvirga (ASV 68, P = <.001), unclassified Methloligellacea (ASV 116, P = 0.03), unclassified Rhodomicrobium (ASV 164, P = <.001), unclassified Acidobacteria (ASV 202, P = 0.01), unclassified Neo-b11 (ASV 355, P = 0.04), unclassified C0119 (ASV 581, P = 0.03), unclassified Planctomycetes (ASV 742, P = 0.04), unclassified Omnitrophicaeota (ASV 1056, P = 0.03), and unclassified Omnitrophicaeota (ASV 3775, P = 0.03). Generally, landraces and wild relatives were found to be enriched with unclassified Methloligellacea (ASV 116), unclassified Rhodomicrobium (ASV 164) and unclassified Planctomycetes (ASV 742) when compared to modern cultivars. While modern cultivars and wild relatives were generally enriched with unclassified Omnitrophicaeota (ASV 3775) when compared to landrace accessions. Lastly, wild relative accessions were generally enriched with unclassified Gemmatimonadetes (ASV 20), unclassified Acidobacteria (ASV 202), unclassified Neo-b11 (ASV 355), unclassified C0119 (ASV 581) when compared to landraces and modern cultivars.

Fungal Communities

For fungal rhizosphere communities, α-diversity, Shannon (P = <0.001, P = 0.001), and Simpson (P = 0.001, P = 0.013) indices were significant for both domestication history and accession,whereas, Fisher (P = 0 .035, P = 0.279) index was only significant for domestication history. Abundance-based coverage estimators (P = 0.692, P = 0.597) and Chao1 (P = 0.692, P = 0.597) indices were non-significant for both domestication history and accession, respectively (Supplemental Figure S2). Additionally, β-diversity (Bray-Curtis dissimilarity) between accessions (PERMANOVA, P = <.001) and domestication history (PERMANOVA, P = <.001) was significant at α = .05. Furthermore, db-RDA analysis revealed that domestication history accounts for 93.7% (RD1 48.2%, RD2 45.5%) of the variation found in the fungal microbiome (Figure 3B).

Lastly, significant differences between accessions for differential abundance were detected for a single ASV, unclassified Mortierella (ASV 11, P = <0.001), with landraces generally having higher enrichment than wild and modern cultivars.

PSF and Rotational Values for Corn Measurements

The PSF values for wild and domesticated peas were widespread with positive, negative, or neutral values (Supplemental Figure S3). Despite the present variation between wild and domesticated peas, rotational values for cob weight ($F_{1,50}$ = 3.036, P = 0.088), vegetative weight (P = 0.355), plant height (P = 0.859), and chlorophyll content for newest (P = 0.567) and oldest (P = 0.729) leaf were non-significant between wild and domesticated peas. Similarly, accession corn PSF values varied with positive, negative, or neutral values. However, rotational values (cob weight) were significantly different between accessions (P = 0.021), with accessions W6 26154 PSP (wild) and PI 577142 (domesticated) having the two highest rotational values (Figure 3). Vegetative weight (P = 0.328), plant height (P = 0.874), and chlorophyll content for newest (P = 0.849) and oldest (P = 0.338) leaf were non-significant between accessions.

Rotational value was significantly correlated with a number of cover cropping measurements. Iron (r =.333, P = 0.014) was the only PSF soil calculation that was positively correlated with rotational value. Additionally, the total aboveground biomass (r =.357, P = 0.007) of the plot was the only pea aboveground measurement significantly correlated with rotational value. Furthermore, the presence of three prokaryotic phyla were significantly positively correlated with rotational value; Gemmatimonadetes (r =.356 , P = 0.008), Armatimonadetes (r =.311, P = 0.022), and Planctomycetes (r =.290, P = 0.033).

Discussion

The main aim of this study was to determine if variation in cover cropping traits and rotational value exists within pea. Our data revealed that variation in cover cropping traits does exist within pea, with significant differences found between modern cultivars, landraces, and wild peas. Furthermore, when focusing on the accession level, significant variation was found in PSF soil measurements and rotational values. Therefore, our results indicate that the genotype of a cover crop could have a profound effect on soil properties and the yield of a subsequently planted crop. However, this study's limitations must be considered, as this experiment took place at a single site over one cover cropping season. Therefore, gene-environment interactions and soil legacy effects, which have been seen to influence plant physiology, could have had an impact on our findings (Detheridge et al., 2016; Huang et al., 2013; Wang et al., 2017). Future multi-site and multi-year trials would be needed to determine whether the results obtained in this study were field-specific or not. Despite these limitations, our findings are novel as they illustrate that crops could be improved as rotational partners, highlighting the use of wild relatives as a phenotypic reservoir for crop improvement.

Plant-soil feedbacks and domestication history:

Plant-soil feedback (PSF) measurements were significantly influenced by domestication, with modern cultivars and landraces, increasing macro- (C% and N%) and micronutrients (manganese) in the soil relative to the control plots (Fig. 1). When focusing on the accession level, significant differences were also observed for macro- (C% and magnesium) and micronutrients (manganese, calcium, and sodium) between accessions (Fig. 2). These results are not surprising since cover cropping pea has been previously shown to increase the presence of macro- and micronutrients in soil, with legumes being proficient at increasing soil N and C (McDaniel et al., 2014). Additionally, Mwafulirwa et al. (2016) noted differences in C deposition for barley genotypes. However, this is the first time—to our knowledge—that differences in these benefits have been described for pea. Overall, these results indicate that pea could be potentially bred to improve its effect on soil properties in agroecosystems.

Recruited prokaryotic communities did not differ in α-diversity between domesticated and wild peas at the history or accession levels. This was expected, as previous studies have shown a nonsignificant difference in α-diversity between CWRs and their domesticated counterparts (Pérez-Jaramillo et al., 2016; 2017). Additionally, β-diversity and differential abundance analysis revealed that pea rhizospheres of domesticated and wild accessions were not significantly different (α = .05) from each other. These results were unexpected, as a previous meta-analysis revealed β-diversity and enrichment differences in differential abundances between wild and domesticated barley (*Hordeum vulgare)*, lettuce (*genus Lactuca*), common bean (*Phaseolus vulgaris*), and hairy bittercress *Cardamine hirsuta* (Pérez-Jaramillo et al., 2017). Pérez-Jaramillo et al. (2017) concluded that wild relatives' rhizospheres were enriched with *Bacteroidetes*, while their domesticated counterparts were enriched with *Actinobacteria* and *Proteobacteria*. The disparity between our study's results and previous findings could stem from differences in environments (Fierer, 2017; Fierer & Jackson, 2006) and land management practices (Qiao et al., 2017), which have been shown to have stronger effects on soil microbial communities than plant genotypes. Additionally, the lack of significance for β-diversity and differential abundances between pea accessions could have resulted from the limited number of accessions used in this study as it may not have fully captured the entire genetic or phenotypic diversity of microbial recruitment in pea.

Despite finding nonsignificant differences for prokaryotic recruitment within pea, we did observe significant differences in α and β-diversity for recruited fungal communities due to domestication and accession. These results are consistent with Chartrel et al. (2021) who found differences in α (Observed and Shannon) and β-diversity (Bray-Curtis dissimilarity) in pea due to country of origin (France, Sweden, Canada, all modern cultivars). Additionally, our results agree with Brisson et al. (2019) and Favela et al. (2021), which revealed that domestication and breeding have impacted Maize rhizosphere microbial community recruitment. In total, our results demonstrate that domestication and breeding have impacted pea rhizosphere fungal communities, and illustrate that wild relatives and landraces can be potentially utilized in breeding programs to improve fungal recruitment in pea (Coyne et al., 2020; Gopal & Gupta, 2016).

Rotational Value

The effect of accession and domestication history of a previously planted pea cover crop on a subsequently planted crop was limited, with nonsignificant differences found for plant height, chlorophyll content, and aboveground biomass. However, pea genotype did significantly influence rotational values (cob weight). Accessions W6 26154 PSP (wild), and PI 577142 (domesticated) had the two highest average rotational values (Fig. 4). This may, in part, be due to these accessions having neutral and the second-highest PSF C% measurements, respectively (Fig. 2). Additionally, accession W6 26157 PSP had the lowest rotational value and the lowest PSF Soil C% measurement. On average, legume cover crops have been shown to increase soil C by 24.5%, the highest soil C increase of all cover crops (Austin et al., in review). Moreover, long-term rotations, including pea and spring wheat rotations, increase total soil C and grain yields more effectively than other rotation combinations (Sainju et al., 2017). More importantly, studies have shown that soil C is positively correlated with yields in agroecosystems (Lal, 2004; Sainju et al., 2017). However, in our study, PSF total soil C% was not positively correlated with rotational value which may be due to the length of our study. Longer implementations of cover crops have been shown to have a more profound effect on soil organic carbon and soil organic matter, which contribute to total soil C% measurements (Olson et al., 2014; Poeplau & Don, 2015). Nonetheless, our results do suggest that the manipulation of soil total C% may have an integral role in determining the rotational value of accessions.

Rotational value was moderately positively correlated with several cover cropping measurements, one of which was the presence of iron. Iron is an essential micronutrient with strong effects on plant growth and yield due to it being a prerequisite for many cellular functions, such as photosynthesis, respiration, enzyme cofactors, redox reagent, and amino acid synthesis (reiewed in Govindaraj et al., 2011; Kumar et al., 2017). Therefore, a correlation between rotational value and iron was not surprising. Additionally, rotational value was moderately positively correlated with the presence of three prokaryotic phyla, *Gemmatimonadetes*, *Armatimonadetes*, and *Planctomycetes*. *Gemmatimonadetes* may increase rotational value by suppressing diseases, as it has been significantly negatively correlated with bacterial wilt infection rates in tomato (Zhang et al., 2020) and *Fusairum* wilt in banana (Fan et al., 2022; Shen et al., 2014). Similarly, several studies have correlated the presence of *Armatimonadetes* with disease suppression. For instance, significant negative correlations between *Armatimonadetes* with disease index of bacterial wilt were observed in tobacco (Chen et al., 2020) and vanilla (Xiong et al., 2015). Furthermore, the relative abundance of *Armatimonadetes* was found to be enriched following yellow mosaic disease infection in wheat (Wu et al., 2021) and was exclusively associated with the rhizosphere of asymptomatic avocado trees in an orchard infected with Fusarium dieback (Bejarano-Bolívar et al., 2021). Despite these relationships between cover cropping measurements and rotational values, our experimental design was unable to determine if these relationships were correlative or causational. Further experimentation that manipulates the absence and presence of these variables is required to evaluate the true relationship between these variables and rotational value.

The importance of Cover Cropping

Cover cropping and crop rotations have been used in numerous agroecosystems throughout agricultural history to improve yields and soil quality. The results obtained from this study highlight the significant impacts of genotype on a cover crop performance. Implications from our research suggest that researchers studying cover cropping may now need to narrow to the genotype level rather than the family level (legumes, cereals, etc.) to facilitate agricultural production. Furthermore, CWRs should be utilized in cover cropping studies to reintroduce lost beneficial phenotypic and genotypic variation. In all, the findings of this study suggest that cover crops can be improved as rotational partners to increase subsequently planted crop yields. As such, improving cover crop rotational values is imperative for sustainable agriculture and meeting the future nutritional needs of a growing human population.

Declarations

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Competing Interests: The authors declare no financial conflicts of interest.

Author Contributions: Edward Marques and Eric von Wettberg conceived the ideas and designed methodology; Edward Marques, Lauren Kerwien, Erika Bueno collected the data; Edward Marques and Erika Bueno analysed the data; Edward Marques, Erika Bueno, and Eric von Wettberg led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data Availability: Data are available on our Open Science Foundation site for this project, https://osf.io/2kbrf/

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Figures

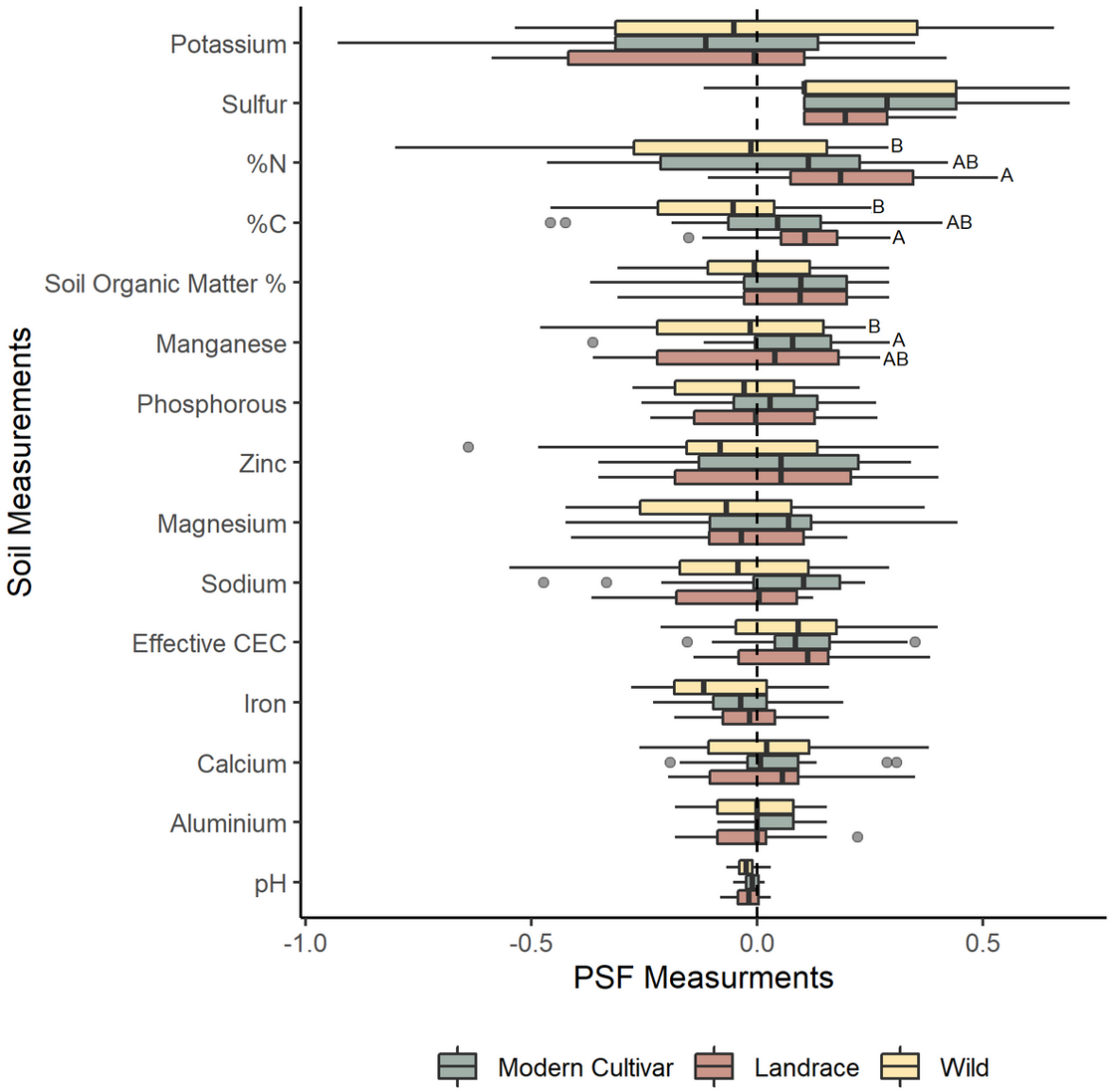

Figure 1

PSF soil measurements by domestication history (Modern Cultivar, Landrace, Wild). Letters indicate significant difference within soil measurement at the $p < 0.05$ level.

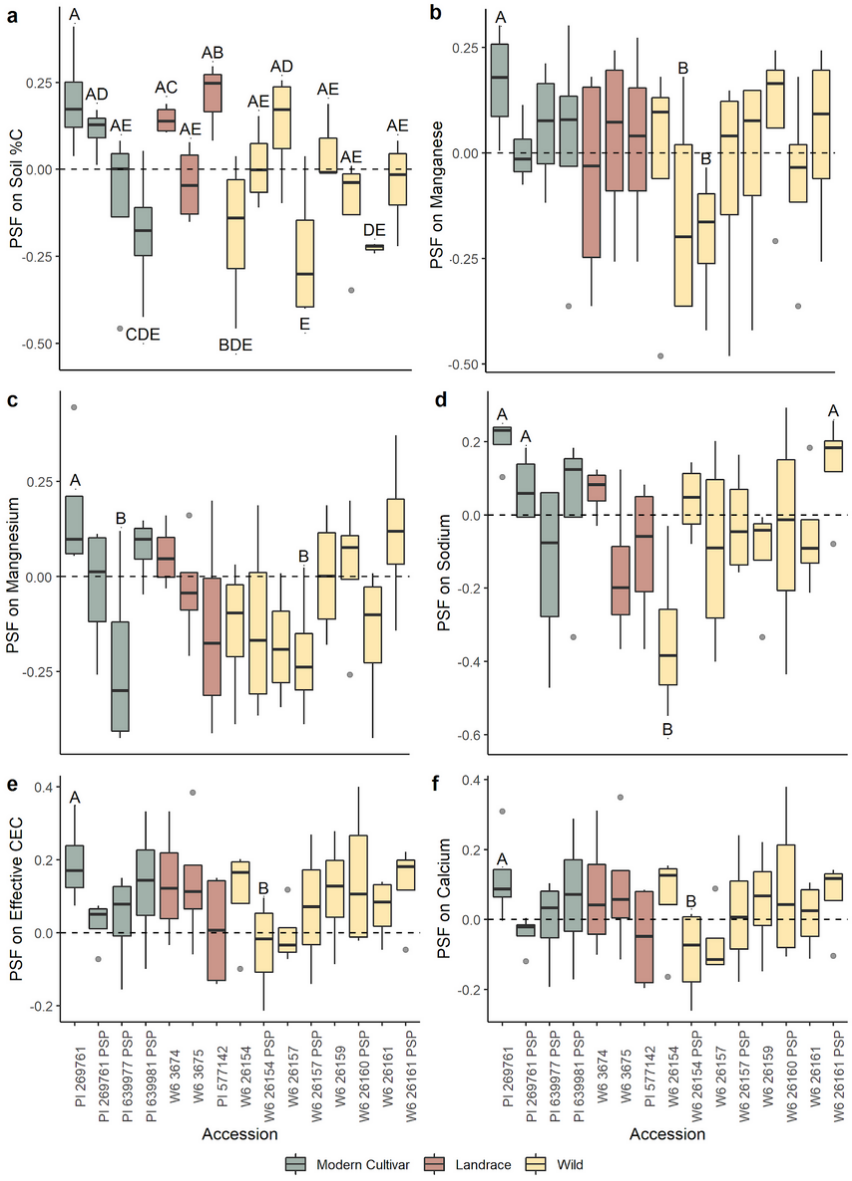

Figure 2

PSF soil measurements by accession (colored by domestication history: Modern Cultivar, Landrace, Wild) for (a) %C, (b) Manganese, (c) Magnesium, (d) Sodium, (e) Effective cation exchange capacity (CEC), (f) Calcium. Letters indicate significant difference within soil measurement at the $p < .05$ level.

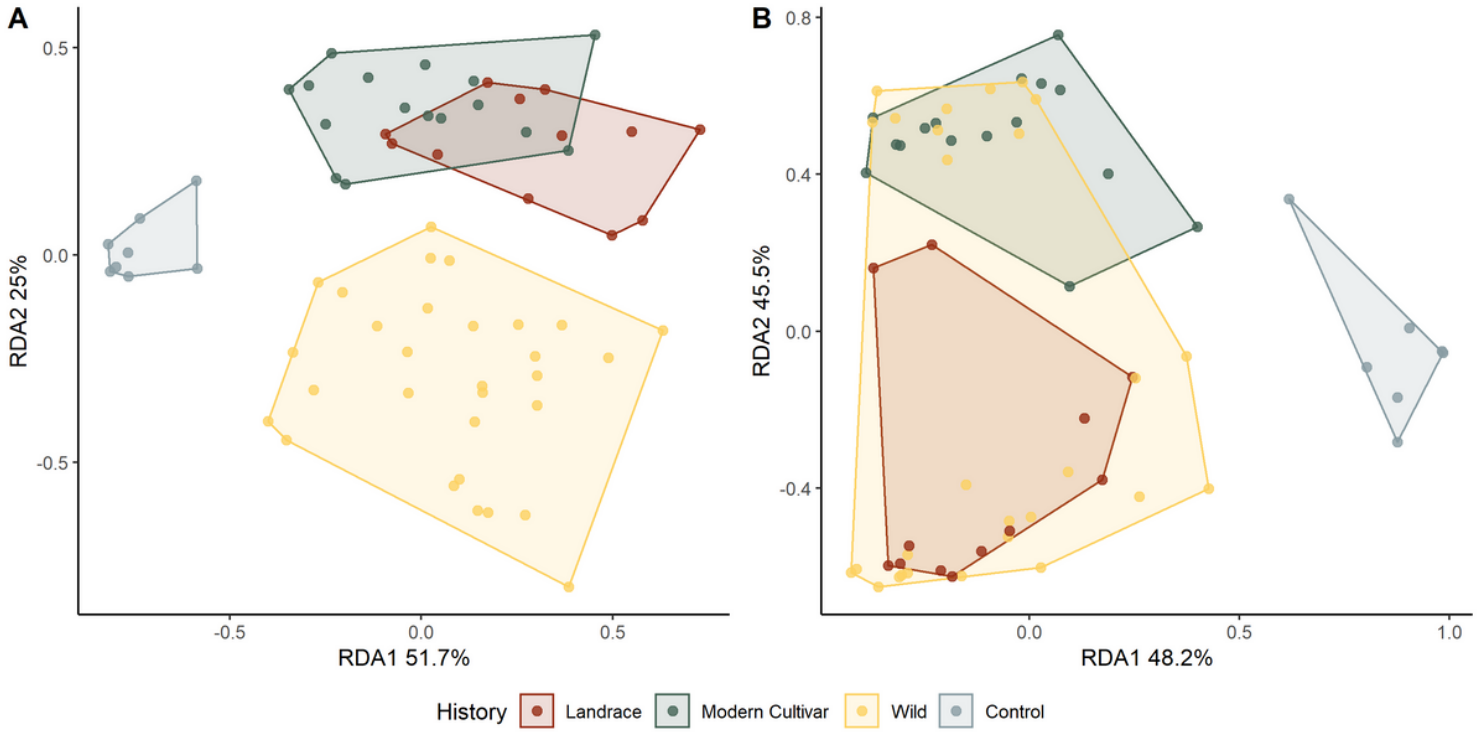

Figure 3

Redundancy analysis of species composition by domestication history (Modern Cultivar, Landrace, Wild, Control) for (A) prokaryotic and (B) fungal communities.

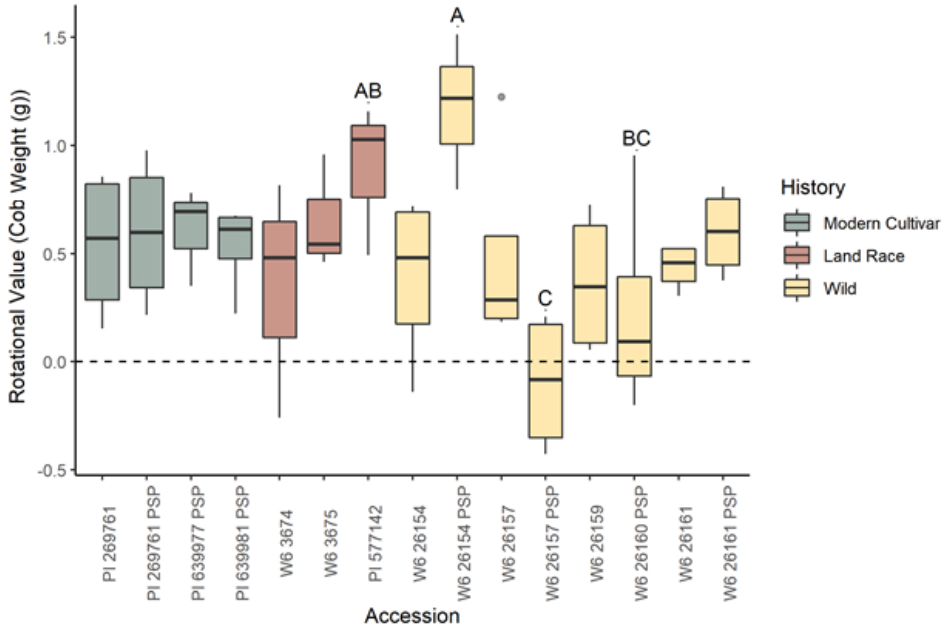

Figure 4

Rotational value measurements by accession (colored by domestication history: Modern Cultivar, Landrace, Wild). Letters indicate significant difference within soil measurement at the $p < 0.05$ level.

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