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Cover Crops and Compost Influence Soil Enzymes during Six Years of Tillage-Intensive, Organic Vegetable Production

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Soil enzymes are considered sensitive indicators of soil health but are not well understood in tillage-intensive vegetable systems. The activities of soil enzymes involved in nutrient cycling (β-glucosidase, β-glucosaminidase, alkaline phosphatase, dehydrogenase, aspartase, and L-asparaginase) were evaluated during 6 yr of commercial-scale production in five organic vegetable systems in Salinas, CA. The systems differed in yard-waste compost inputs (none or 15.2 Mg ha^{-1} yr^{-1}), winter cover crop frequency (annually or every fourth year), and cover crop type (legume-rye, mustard, or rye). Large differences in cumulative organic matter input (7.4 to 136.8 Mg ha^{-1}) from compost and cover crop shoots affected soil enzyme activities. With exception of aspartase, all enzyme activities were on average lowest without compost, intermediate with compost and infrequent cover cropping, and highest with compost and annual cover cropping. After 6 yr of vegetable production there was a positive relationship between microbial biomass and activities of all enzymes except aspartase. Despite lower inputs of cover crop shoot biomass from mustard compared with rye and the legume-rye, and differences in shoots residue quality, cover crop type had relatively little influence on enzyme activities. We conclude that soil enzyme activities were influenced primarily by annual cover cropping. These results and other attributes of soil health in this long-term study illustrate the importance of frequent cover cropping in tillage-intensive vegetable production. This raises questions about the sustainability of organic and conventional vegetable systems if cover crops are seldom used, and highlights the need for innovative strategies to increase cover cropping.

Abbreviations: CI, confidence interval; ESCI, Exploratory Software for Confidence Interval; INT, 2-(4-iodophenyl)3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

Core Ideas

- **There were large differences in soil enzyme activities within organic systems.**
- **Compost and annual cover crops increased activities of most soil enzymes.**
- **Activities of most soil enzymes were correlated with microbial biomass.**
- **Cover crop type did not influence soil enzyme activities.**
- **The results show the soil health benefits of frequent cover crops in vegetable systems**

Enzymes are complex macromolecules that help organisms—from microscopic bacteria to gigantic whales and long-lived trees—to achieve essential biological processes by accelerating chemical reactions. In the soil, enzymes play critical roles in microbially mediated transformations of organic matter and nutrient cycling. For more than 50 yr, soil enzyme activities have interested scientists and have provided insights into the effects of soil management practices such as organic matter and fertilizer inputs, tillage, and crop rotation (Balota et al., 2014; Bolton et al., 1985; Dick, 1994; Ekenler and Tabatabai, 2003; Elfstrand et al., 2007; Ladd, 1978; Martens et al., 1992). In essence, soil enzymes along with other soil attributes contribute to what has eloquently been called the soil's 'memory' (Janzen, 2016) and which has the potential to help attentive farmers and scientists develop more sustainable farming systems.

The scientific literature on soil enzymes in vegetable, field production systems is relatively limited and includes both experimental studies (Bandick and Dick,

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1999; Hamido and Kpomblekou-A, 2009; Karasawa et al., 2015; Mendes et al., 1999; Miller and Dick, 1995; Pritchett et al., 2011) and observational studies (Acosta-Martinez et al., 2008; Bowles et al., 2014; Kremer and Hezel, 2013; Moeskops et al., 2010, 2012; Sotomayor-Ramirez et al., 2009; Wang et al., 2016); there is also some soil enzyme work from greenhouse or polytunnel systems for vegetable production (Bonanomi et al., 2014; Hernandez et al., 2016; Morra et al., 2010; Shen et al., 2010). While several of these field studies have provided valuable information on the potential benefits of cover crops on soil biology in vegetable systems (Bandick and Dick, 1999; Hamido and Kpomblekou-A, 2009; Mendes et al., 1999; Miller and Dick, 1995; Pritchett et al., 2011), the crop rotations evaluated were far less tillage-intensive than the typical, high-input, vegetable systems in the Salinas Valley of California. This region produces a large portion of the organic and conventional vegetables in the United States, and farms here usually need to produce two or more vegetable crops per field annually to be profitable. This production intensity complicates the adoption of best management practices (i.e., winter cover cropping) to reduce nutrient losses, and explains why many farmer in this region prefer to use yard-waste compost to add large amounts of organic matter to the soil (Brennan, 2017b, 2018a; Hartz, 2006). However, evaluations of the soil food web using nematode community analysis (Ferris et al., 2012) and microbial biomass (Brennan, 2018b; Brennan and Acosta-Martinez, 2017) in a long-term study with high-input, tillage-intensive organic vegetable production in the Salinas Valley showed that frequent cover cropping is the primary management practice driving improvements in the soil food web. This highlights the need to develop novel strategies to increase the adoption of cover cropping in intensive vegetable production systems (Brennan, 2017a, 2017b).

Our objective in this paper is to augment previous reports (Brennan and Acosta-Martinez, 2017, microbial community size and composition; Ferris et al., 2012, nematode community) on soil food web and soil health changes that occurred during the first 6 to 8 yr of a long-term systems study by evaluating the impact of winter cover cropping frequency (i.e., annually versus every fourth year), cover crop type (legume-rye, rye, mustard), and yard-waste compost on the activities of several soil enzymes. These enzymes are involved in biogeochemical cycling of C (β-glucosidase, dehydrogenase), C and N (β-glucosaminidase), N (aspartase and L-asparaginase), and P (alkaline phosphatase). This approach enabled us to identify links between specific soil enzymes and other soil microbial attributes across a range of certified organic systems that differed in the quality and quantity of organic matter inputs from cover crops and compost. To our knowledge, this is the first paper to characterize changes in soil enzymes in a tillage-intensive vegetable production system experiment in California.

MATERIALS AND METHODS

Site Characteristics, Climate, Management, and Experimental Design

A more detailed description of the field site and management, and tillage of the ongoing Salinas Organic Cropping Systems (SOCS) experiment is in Brennan and Boyd (2012a) and Brennan and Acosta-Martinez (2017) and thus will only be described briefly. The experiment is located at the USDA-ARS organic research farm (lat. 36.622658, long. −121.549172, elevation 37 m) in Salinas, CA, approximately 25 km inland from Monterey Bay. The average air temperature from 2003 to 2009 was 11°C from October to March when the cover cropping or winter fallows occurred, and 15°C during the most typical vegetable production period (April to September) (www.cimis.water.ca.gov, Station #89, South Salinas). The average annual rainfall from 2003 to 2009 was 285 mm and occurred mostly between October and March. The soil is a Chualar loamy sand (fine-loamy, mixed, thermic Typic Argixerol). From 1990 to 2003 prior to the SOCS experiment, the field was used for hay, vegetable, and sugar beet trials with occasional winter cover crops, frequent winter fallows, and received minimal inputs of fertilizers or compost. The field has been certified organic by California Certified Organic Farmers since 1999, and to USDA National Organic Program standards since they were implemented in 2002.

The experimental design was a randomized complete block with eight systems in four blocks, but only five systems with optimal, cover crop seeding rates for weed suppression (Brennan, unpublished data) were included in the current paper (Table 1). System plots were 19.5 m long and 12.2 m wide. These five systems of interest were the same ones used for the analysis of soil microbial biomass (Brennan and Acosta-Martinez, 2017) whereas in the analysis of the soil nematode community (Ferris et al., 2012), all eight systems were included with the data pooled across seeding rates. The five systems differed in organic matter inputs from cover crops shoots and yard-waste compost over the 6 yr, ranging from System 1 with the least inputs (7.4 Mg ha^{-1}; oven-dry weight basis) from a single cover crop to System 3 that received the most inputs (45.6 Mg ha^{-1} from cover crop shoots and 91.2 Mg ha^{-1} from yard-waste compost). Table 1 indicates the systems that were used to evaluate the three experimental factors of interest (compost, cover cropping frequency, and cover crop type).

The experiment began with either a winter fallow (Systems 1 and 2) or winter cover crop (Systems 3, 4, and 5) in October, 2003. The cover crops were planted as a solid stand with a grain drill. During the first 6 yr, Systems 1 and 2 were fallow all winter except the fourth winter (2006 to 2007), when they were cover cropped with the legume-rye mixture; Systems 3, 4, and 5 were cover cropped every winter. Cover crops were incorporated with a soil spader in February or March prior to bed formation; the bare winter fallowed systems were also spaded at this time. In Systems 1 and 2 with infrequent winter cover crops, weeds were controlled regularly as needed during the winter fallow by hand weeding or flaming, and shallow tillage; other than the occasional shallow tillage to control weeds during the fallow periods in Systems 1 and 2, the same level of intensive tillage (i.e., spading, bed shaping, weed cultivation, deep ripping) was applied across all systems. After a cover crop decomposition period of 30 to 72 d (depending on rainfall), 101.6-m wide beds were formed with a tractor. The yard-waste compost (7.6 Mg ha^{-1} oven-dry weight,

Table 1. Description of experimental factors, and organic matter inputs from winter cover cropping and compost in five systems that were evaluated for soil enzymes changes over 6 yr in the Salinas Organic Cropping Systems experiment.

System ID	Cover crop		Experimental factors and systems used to evaluate each factor			Organic matter input†		
	Type	Frequency	Yard-waste compost	Cover crop frequency	Cover crop type	Yard-waste compost	Cover crop shoots	Total
						Mg ha^{-1}		
1	Legume-rye‡	Every fourth year	X			0	7.4	7.4
2	Legume-rye‡	Every fourth year	X	X		91.2	8.0	99.2
3	Legume-rye‡	Annually		X	X	91.2	45.6	136.8
4	Mustard§	Annually			X	91.2	30.9	122.1
5	Rye¶	Annually			X	91.2	43.1	134.3

† Organic matter inputs over the 6 yr are on an oven-dry weight basis. Yard-waste compost was applied at 15.2 Mg ha annually in all systems except System 1.

‡ By seed weight, the legume-rye mixture included 10% rye ('Merced' *Secale cereale* L.), 35% faba bean (*Vicia faba* L.; small-seeded type known as 'bell bean'), 25% pea ('Magnus' *Pisum sativum* L.), 15% common vetch (*V. sativa* L.), and 15% purple vetch (*V. benghalensis* L.) and was planted at 420 kg ha^{-1}.

§ Mustard was a mixture of 61% white mustard ('Ida Gold' *Sinapis alba* L.) and 39% India mustard ('Pacific Gold' *Brassica juncea* L. [Czern.]) and was planted at 11 kg ha^{-1}.

¶ Rye ('Merced' *Secale cereale* L.) was planted at 90 kg ha^{-1}.

C to N ratio of ~22) was applied to the peaked bed tops of all systems except System 1, and supplemental pelleted organic fertilizer (56 to 66 kg N ha^{-1}) was injected into the beds that were then shaped to incorporate the compost and create a flat bed top for transplanted lettuce which was the first of two vegetables each year; additional liquid organic fertilizer was applied to the lettuce by drip irrigation to bring the total N application rate up to 73 kg ha^{-1}. The lettuce was planted in May or June and harvested after approximately 45 d and was followed by spinach (July to August, 2004) or transplanted broccoli (July to September/October, 2005 to 2009). Yard-waste compost (7.6 Mg ha^{-1}) was applied to all systems (except System 1) before all vegetable crops to achieve an annual compost application rate of 15.2 Mg ha^{-1}. The total N rates from supplemental fertilizers did not differ between systems (22 kg ha^{-1} for spinach; 56 to 66 kg N ha^{-1} for lettuce; 134 to 170 kg N ha^{-1} for broccoli). Beds for broccoli were the same width as for lettuce, but were twice as wide for spinach. Irrigation (sprinkle and/or drip) was uniformly applied across all systems during vegetable production, and sprinkle irrigation was also used to establish the cover crops before the winter rainfall began. Intensive tillage was used as needed between vegetables and following the commercial-scale harvest of the marketable vegetables with collaborating local farms (Brennan and Boyd, 2012a).

Soil Sampling and Assays of Soil Enzyme Activity

Soil samples for analysis of enzyme activities for Time 0 and after 6 yr were collected as described previously (Brennan and Acosta-Martinez, 2017) from the four replicates for each system in October from bare, flat plots (i.e., without beds) that had all been uniformly tilled to at least 30 cm depth with a disc harrow, chisel or spader to prepare the plots for winter cover crops or fallow. Briefly, the samples were collected from six to eight cores that were 6.7-cm diameter to a depth of 6.5 cm that were mixed and frozen at –25°C.

Soil enzymes activities were determined between 2009 and 2010 as follows. All samples were assayed in duplicates and included controls for each assay where the substrate was added after the reaction was stopped following incubation. Our analysis of changes in soil enzyme activities from time zero (2003) to 6 yr later focused on two glycosidases (β-glucosidase, β-glucosaminidase) and a phosphomonoesterase (alkaline phosphatase) determined according to Tabatabai (1994) or Parham and Deng (2000) for β-glucosaminidase. Enzyme activities were assayed using 0.5 g of air-dried soil under a final concentration of 10 mM of the specific enzyme substrate (p-nitrophenyl-derivate), optimal pH and buffer (without toluene), and incubated for 1 h at 37°C. The concentration of the reaction product (p-nitrophenol) was determined colorimetrically at 400 nm using a spectrophotometer (Beckman Coulter DU640, Brea, CA).

The activities of two amidohydrolases (aspartase, L-asparaginase) and dehydrogenase were also evaluated but only in soil collected after 6 yr of the experiment. The assay for the amidohydolases (Tabatabai, 1994) involved steam distillation using a Foss Kjeltec 2200 Auto Distillation Unit (Foss North America, Eden Prairie, MN) to collect the product of reaction into the distillate (release of amide and converted into ammonia and/or ammonium) and titration with a Mettler Toledo DL 50 titrator (Mettler-Toledo Inc., Columbus, OH). The dehydrogenase assay was determined in field moist soil and results are expressed in micrograms of 2-(4-iodophenyl)3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) per gram of dry soil per hour, as described in Prosser et al. (2011).

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987) and was described in detail in Brennan and Acosta-Martinez (2017).

Statistical Analyses and Data Presentation

The data analysis focused on using point and interval estimates to make statistical inferences and determine the practical significance of the results. The point and interval estimates used were mean paired differences (i.e., effect sizes) and their 95% confidence interval (CI). The CIs were calculated in SAS

(ver. 9.4) (SAS Ins. Cary, NC) using PROC MEANS and in the Exploratory Software for Confidence Interval (ESCI) (Cumming and Calin-Jageman, 2017). The CIs are reported in square brackets [ ] in the text. We chose this analysis approach based on valid criticisms of null-hypothesis significance testing (Anderson et al., 2000; Campbell et al., 2015; Fidler et al., 2006; Hubbard and Lindsay, 2008; Lambdin, 2012; Nakagawa and Cuthill, 2007) that can lead to misinterpretations of results and dichotomous thinking. To evaluate the effects of the three experimental factors of interest (i.e., compost, cover cropping frequency and type), two basic types of paired comparisons were made including: (i) comparisons of changes in enzyme activity within a system from Time 0 to after 6 yr (i.e., a repeated measure), and (ii) comparisons in enzyme activity between pairs of systems after 6 yr of management. For changes in enzyme activity over time, we calculated the difference in enzyme activity from Time 0 to after 6 yr within each replicate and then calculated the CI of the mean paired difference within each system. Similarly, for the between-systems comparisons of the enzyme activities measured after 6 yr alone, we calculated the difference in the enzyme activity between the two systems within each replicate, and then calculated the CI of the mean paired difference.

The paired comparisons within a system from Time 0 to after 6 yr were used to evaluate changes in the activities of β-glucosidase, β-glucosaminidase, and alkaline phosphatase, whereas with the other enzymes (aspartase, L-asparaginase and dehydrogenase) we were only able to make comparisons between systems after 6 yr because Time 0 measurements were not made. We assumed that the comparisons from Time 0 to after 6 yr would provide a more precise measure of the effects of the experimental factors on enzyme activity (β-glucosidase, β-glucosaminidase, and alkaline phosphatase) because they represent changes within the experimental unit (i.e., system plot within each replicate) over time. The comparisons between two systems for the other enzymes (aspartase, L-asparaginase and dehydrogenase) after 6 yr alone are considered paired comparisons because the systems are paired within each block in the randomized complete block design.

With both of these types of paired data, inferences should be made based on the mean paired difference and the CI of this mean difference (Cumming and Finch, 2005). The location of a mean difference and its CI, relative to zero, can be used to evaluate the evidence of a true effect whereby effect sizes and CIs that are further from zero provide more evidence of a true difference. If the CI of a paired difference does not include zero than $P < 0.05$ (i.e., a comparison-wise error rate < 5%) and $P = 0.05$ if one of the limits is just zero. We mention the relationship between P values and CIs as a point of reference that readers may be more familiar with, but we discourage readers from using CIs in a rigid or dichotomous way; for example, by concluding that a CI of a paired difference that includes zero indicates no difference. Rather, we suggest readers consider: (1) the patterns in the raw data, (2) the direction and size of the mean effect and width of the CI, and (3) visualize CIs in the shape of a 'cat's eye' (Supplemental Fig. S1). Cat's eye CIs illustrate that values are most plausible at the 'fattest' part of the CI in the center near the mean, and gradually become less plausible as the cat's eye narrows toward the upper and lower limits (Cumming, 2012); cat's eye CI pictures are a promising and intuitive tool to improve understanding of CIs (Kalinowski et al., 2018). Supplemental Fig. S2 through S10 provide additional detailed information using ESCI of the paired comparisons of interest and may be helpful to readers that are unfamiliar with this estimation approach for making statistical inferences; all of the raw data on microbial biomass and enzyme activities from the five systems described in the present paper and three additional systems in the study are available in Brennan and Acosta-Martinez (Brennan and Acosta-Martinez, 2018).

For the various comparisons, the effect sizes of changes in enzyme activity over time or of differences in enzyme activities between systems are provided in the original units of enzyme activity in the figures, and were also calculated as a standardized effect size measure (Cohen's unbiased d, hereafter "d_{unb}"). Cohen's d_{unb} was calculated in ESCI by dividing the effect size in original units by a standardizer that is based on the standard deviations of the paired two measurements and multiplied by an adjustment factor (Cumming, 2012, p. 294). This standardized effect size can be used to compare the effect size regardless of differences in the scale of the units of measurement.

To help us and readers understand the patterns in our data (variability, skewness and scatter), we plotted the raw data with their CIs as suggested by Drummond and Vowler (2011). SigmaPlot (version 13, Systat Software, Inc., San Jose, CA) and PROC REG in SAS were used to determine and plot the best fit regression relationships between enzyme activity and MBC and MBN. Regression model selection was done to maximize the adjusted R^2 for the best fit first, second, or third order model using the Selection = ADJRSQ option in PROC REG.

RESULTS

Glycosidases (C and/or N Cycling)

At the beginning of the experiment in 2003 (Time 0) before the treatments were imposed, the average activity of β-glucosidase among systems ranged from 57 to 76 mg p-nitrophenol kg soil^{-1} h^{-1}, and after 6 yr the lower average was similar (55 mg kg^{-1} h^{-1}, System 1) while the upper average was considerably higher (118 mg kg^{-1} h^{-1}, System 4) (Fig. 1A). The change in β-glucosidase activity was most apparent in Systems 3, 4, and 5 that were cover cropped annually and showed average increases of 34 to 57 mg p-nitrophenol kg soil^{-1} h^{-1}, and which corresponds to increases of 66 to 100% (Fig. 1B). Among these three annually cropped systems, System 3 was the only one where the CI of the difference included zero [CI = −2, 71] although the raw data for all four replicates showed positive changes in β-glucosidase overtime (Fig. 1B; Supplemental Fig. S2 panels C–E). However, there was some evidence of a greater change in mustard (System 4) than with the legume-rye (System 3) or rye (System 5). For example, note that mustard was the only cover crop where the mean change and change in three of the four replicates exceeded 55 mg p-nitrophenol kg soil^{-1} h^{-1}.

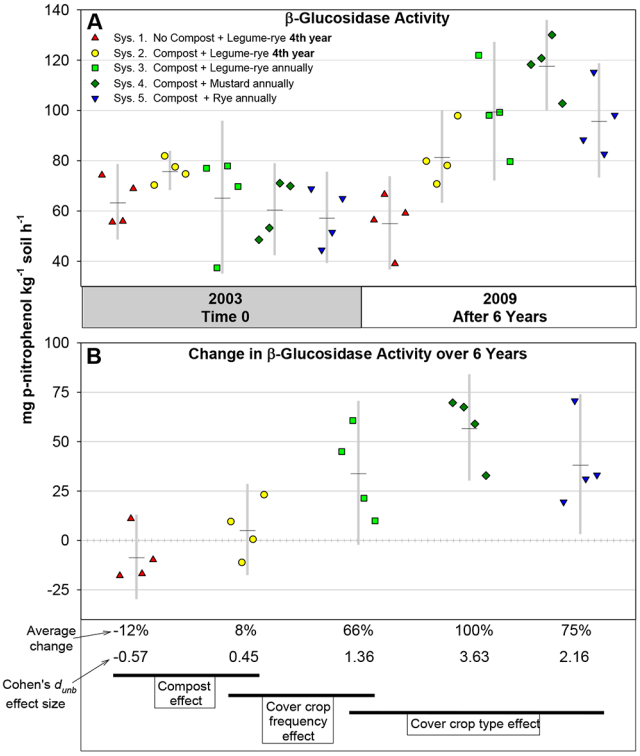

Fig. 1. Beta-glucosidase activity at Time 0 and after 6 yr (A), and the change over 6 yr (B) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4. The percentage change in enzyme activity and the standardized effect size (Cohen's unbiased *d*) from Time 0 to after 6 yr are shown below the *x* axis of panel B. The solid, horizontal lines below the *x* axis in panel B indicate the systems to evaluate the effects of the experimental factors (compost, cover crop frequency and type). See Supplemental Fig. S2A–E for additional details of the paired comparisons (i.e., changes from Time 0 to after 6 yr).

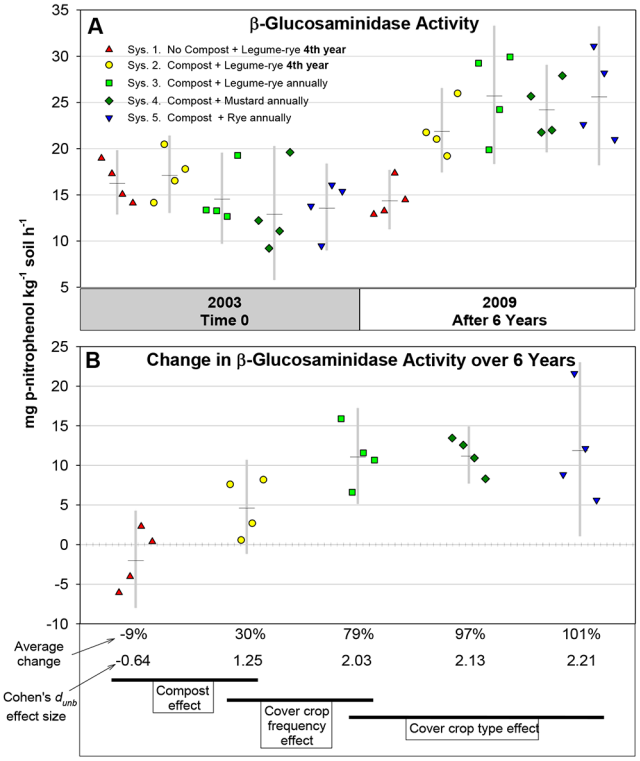

Fig. 2. Beta-glucosaminidase activity at Time 0 and after 6 yr (A), and the difference (B) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4. The percentage change in enzyme activity and the standardized effect size (Cohen's unbiased *d*) from Time 0 to after 6 yr are shown below the *x* axis of panel B. The solid, horizontal lines below the *x* axis in panel B indicate the systems to evaluate the effects of the experimental factors (compost, cover crop frequency and type). See Supplemental Fig. S3A–E for additional details of the paired comparisons (i.e., changes from Time 0 to after 6 yr).

This higher average change in System 4 than in Systems 3 and 5 is also apparent in the higher standardized effect size in System 4 (3.63 mg kg^{-1} h^{-1}) than for System 3 (1.36 mg kg^{-1} h^{-1}) or System 5 (2.16 mg kg^{-1} h^{-1}). However, the data indicate that cover crop type had relatively little effect on this change in β-glucosidase activity overtime (Fig. 1B; Supplemental Fig. S2 panels C–E).

Within Systems 1 to 3 that received the legume-rye cover crop mixture, β-glucosidase activity (mg p-nitrophenol kg soil^{-1} h^{-1}) over the 6 yr on average was negative for System 1 (−8 [CI = −30, 13], −12% change), and increased a small amount for System 2 (6 [−18, 28], +8% change), and increased the most in System 3 (34 [−2, 71], +66% change) (Fig. 1B; Supplemental Fig. S2 panels A–C). This order parallels the inputs of organic matter from cover crop shoots and compost that were 7.4, 99.2, and 136.8 Mg ha^{-1} for Systems 1, 2, and 3, respectively (Table 1). Overall, the scatter of the raw data and the proximity of the CIs of the change over time to zero suggests more similarities between System 1 and 2 that differed in compost inputs, than between Systems 2 and 3 that differed in cover cropping frequency (Fig. 1B; Supplemental Fig. S2 panels A–C).

The activity of β-glucosaminidase at Time 0 ranged from a mean of 13 to 17 mg p-nitrophenol kg soil^{-1} h^{-1} with a large amount of overlap in the CIs of all systems, and after 6 yr ranged from a mean of 14 mg kg^{-1} h^{-1} (System 1) to 22 to 26 mg kg^{-1} h^{-1} for Systems 2 to 5 that all received compost (Fig. 2A). The CI of the change in β-glucosaminidase activity included zero for Systems 1 [−8, 4] and System 2 [−1, 11], although most of the CI of System 2 was positive and had an average increase of 30%; there was a 9% average decrease for System 1. In contrast, the activity of the annually cover cropped systems (Systems 3 to 5) increased by an average of 79% or more after 6 yr and the CIs of the difference did not include zero in these three systems (Fig. 2B; Supplemental Fig. S3 panels C–E). The mean decrease in β-glucosaminidase activity after 6 yr in System 1 was consistent with the decline in β-glucosidase activity although with both enzymes the close proximity of the mean to zero indicates no change overtime. Furthermore the relatively larger changes over the 6 yr in β-glucosaminidase activity in System 3 to 5 than in

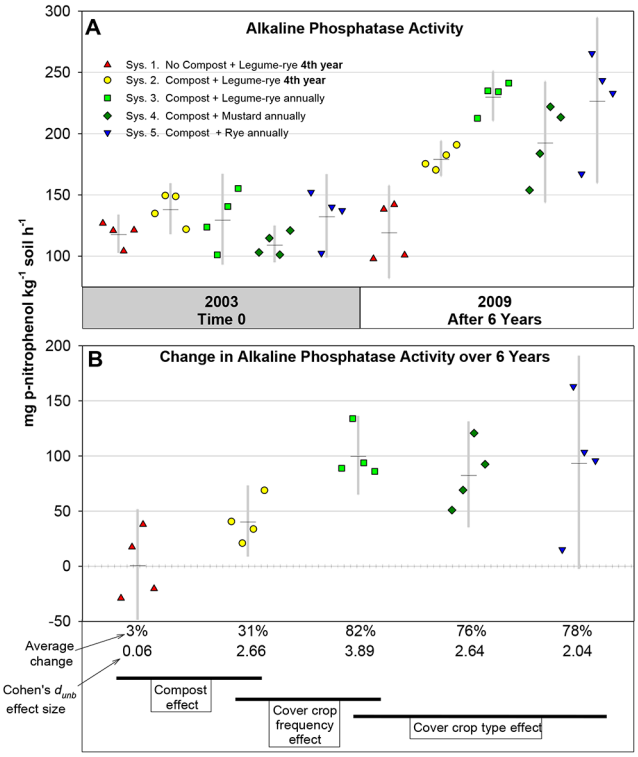

Fig. 3. Alkaline phosphatase activity at Time 0 and after 6 yr (A), and the difference (B) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4. The percentage change in enzyme activity and the standardized effect size (Cohen's unbiased *d*) from Time 0 to after 6 yr are shown below the *x* axis of panel B. The solid, horizontal lines below the *x* axis in panel B indicate the systems to evaluate the effects of the experimental factors (compost, cover crop frequency and type). See Supplemental Fig. S4A–E for additional details of the paired comparisons (i.e., changes from Time 0 to after 6 yr).

System 2 are consistent with the changes that occurred with activity of β-glucosidase. Among the annually cover cropped systems (Systems 3 to 5) the increases in β-glucosaminidase activity overtime were relatively consistent across the three cover crop types although the change was least variable in System 4 that received the mustard cover crop. Among the three legume-rye systems (Systems 1 to 3) the changes in β-glucosaminidase activity over time followed the same basic pattern with β-glucosidase where activity increased with organic matter inputs in order of System 1 < System 2 < System 3. However, there was more evidence that compost additions to System 2 increased the activity of β-glucosaminidase (Fig. 2B) than β-glucosidase (Fig. 1 B); this can been seen by comparing the percentage of change and standardized effect size (d_{unb}) that were at least two-fold greater with β-glucosaminidase than β-glucosidase.

Alkaline Phosphatase

Alkaline phosphatase activity at the start of the experiment ranged from an average of 110 to 139 mg p-nitrophenol kg soil^{-1} h^{-1} (Fig. 3A). After 6 yr there was essentially no change in alkaline phosphatase activity in System 1 that received the least organic matter inputs, an average increase of 41 mg [CI = 8, 73] in System 2 that received compost with infrequent cover cropping, and an average increase of 83 to 101 mg in the annually cover cropped Systems 3, 4, and 5 that received compost (Fig. 3A and B; Supplemental Fig. S4 panels A–E). In the three annually cover cropped systems, all replicates except for the first replicate in System 5, showed increased alkaline phosphatase activity of 50 mg or more (Fig. 3B). As with the glycosidases, in the four systems receiving compost, the percentage change in alkaline phosphatase was considerably greater with annual cover cropping. While the change in alkaline phosphatase activity was more variable in System 5 with rye cover crops, suggesting more uncertainty with this system, the majority of data for these three systems indicates that the activity of this enzyme was not affected by cover crop type (Fig. 3B; Supplemental Fig. S4 panels C–E).

Amidohydrolases (N cycling)

In contrast to all of the other enzymes measured in this study, the average activity of aspartase after 6 yr of management was nearly identical in Systems 2 and 3 that differed only on cover cropping frequency (Fig. 4A and B). Furthermore, among the annually cover cropped systems, aspartase levels were lower by an average of approximately 40 to 50 mg p-nitrophenol kg soil^{-1} h^{-1} in System 5 (rye) than in System 3 (legume-rye), and in System 4 (mustard) than in System 3 (Fig. 4B; Supplemental Fig. S8 panels B and C); however, the scatter of the raw data, and CIs of the differences of the paired comparisons between these annually cover cropped systems indicate considerable uncertainty in whether cover crop type affected asparatase activity. On the other hand, compost was the only experimental factor that had a clear effect on aspartase activity as indicated by an average increase of 85 [43, 127] mg p-nitrophenol kg soil^{-1} h^{-1} in System 2 that received compost versus System 1 that did not.

The activity of L-asparaginase was among the lowest enzyme levels that were measured in the study but followed a similar pattern to that for the glucocidases and alkaline phosphatase with gradual average increases from Systems 1 to 2 to 3 (Fig. 5A). While the CIs of the paired comparisons between these three legume-rye systems all included zero, there is more evidence that compost affected L-asparaginase activity than cover cropping frequency; note the smaller overlap with zero of the CI of comparison of System 1 and 2, than for the comparison of System 2 and 3, and the larger standardized effect size (Cohen's d_{unb}) for the first (2.15) than second comparison (0.89) (Fig. 5B; Supplemental Fig. S6 panels A and B). Among the annually cover cropped systems there was no clear evidence that cover crop type affected L-asparaginase activity (Fig. 5; Supplemental Fig. S9 panels A–C).

Dehydrogenase (C cycling)

On average, dehydrogenase activity after 6 yr increased incrementally with increasing organic matter inputs from System

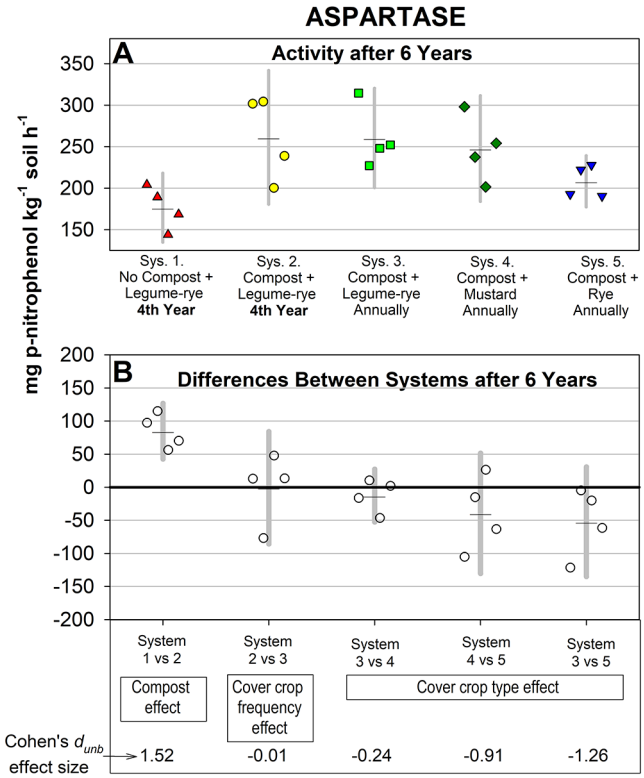

Fig. 4. Activity of aspartase after 6 yr (A) and the differences of paired comparisons between systems after 6 yr (B) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4. The standardized effect size (Cohen's unbiased *d*) is shown below each paired comparison. The boxes below the *x* axis in panel B indicate the system comparisons to evaluate the effects of the experimental factors (compost, cover crop frequency and type). See Supplemental Fig. S5A–B and S8A–C for additional details of the paired comparisons between systems.

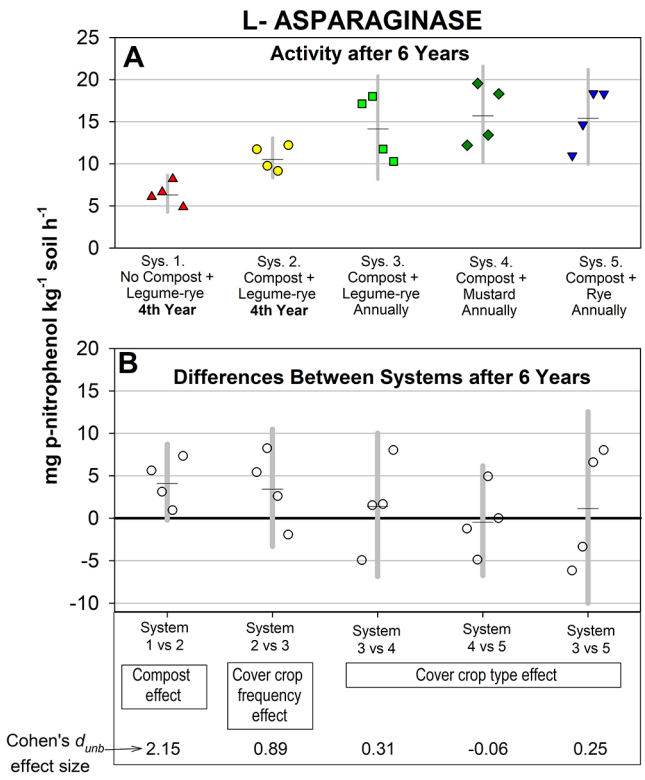

Fig. 5. Activity of L-asparaginase after 6 yr (A) and the differences of paired comparisons between systems after 6 yr (B) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4. The standardized effect size (Cohen's unbiased *d*) is shown below each paired comparison. The boxes below the *x* axis in panel B indicate the system comparisons to evaluate the effects of the experimental factors (compost, cover crop frequency and type). See Supplemental Fig. S6A–B and S9A–C for additional details of the paired comparisons between systems.

1 to 2 to 3 (Fig. 6A). Although the paired comparisons between these three systems both included zero, the CI of these differences and raw data provide more evidence that dehydrogenase activity (μg INT g dry soil^{-1} h^{-1}) was increased by annual cover cropping [−0.1, 3.4] than by compost [−2.9, 5.9] (Fig. 6B; Supplemental Fig. S7 panels A and B); note the wider scatter of the raw data of the paired differences in the comparison of System 1 and 2, than in the comparison of System 2 and 3. Among the three annually cover cropped systems, dehydrogenase activity was greatest on average in System 5 (rye) and lowest in System 4 (mustard) but the majority of the replicate data points and large amount of overlap with zero of the CIs of the paired comparisons between these systems indicate that cover crop type had relatively little consistent effect on the activity of this enzyme (Fig. 6A and B; Supplemental Fig. S10 panels A–C).

Relationship between Soil Microbial Biomass and Soil Enzyme Activities

The range of microbial biomass carbon (MBC) at Time 0 was from 47 to 123 mg C kg soil^{-1} compared with after 6 yr when it was from 62 to 249 mg C kg soil^{-1} (Fig. 7). For the three enzymes (β-glucosidase, β-glucosaminidase, and alkaline phosphatase) that were measured at Time 0 and after 6 yr, there was no evidence of a relationship between microbial biomass carbon (MBC) and enzyme activities at Time 0 for β-glucosidase and alkaline phosphatase (Fig. 7A and E), and only weak evidence of a positive linear correlation for β-glucosaminidase (r^2 = 0.19, P = 0.06; Fig. 7C). As expected at Time 0 before the cover crop and compost treatments were imposed, the enzyme activities and MBC levels were randomly distributed among the system replicates. However, by 6 yr later the data points for the frequently cover cropped systems had increased for MBC and enzyme activities and there were positive relationships between MBC and the activity of β-glucosaminidase, β-glucosaminidase, alkaline phosphatase (Fig. 7B, D, and F). It is striking to notice that the lower end of the fitted curves after 6 yr were due to relatively little change over time for System 1 and 2 that were infrequently cover cropped; only one replicate of System 2 that received compost annually occurred among the data cluster for annually cover cropped systems.

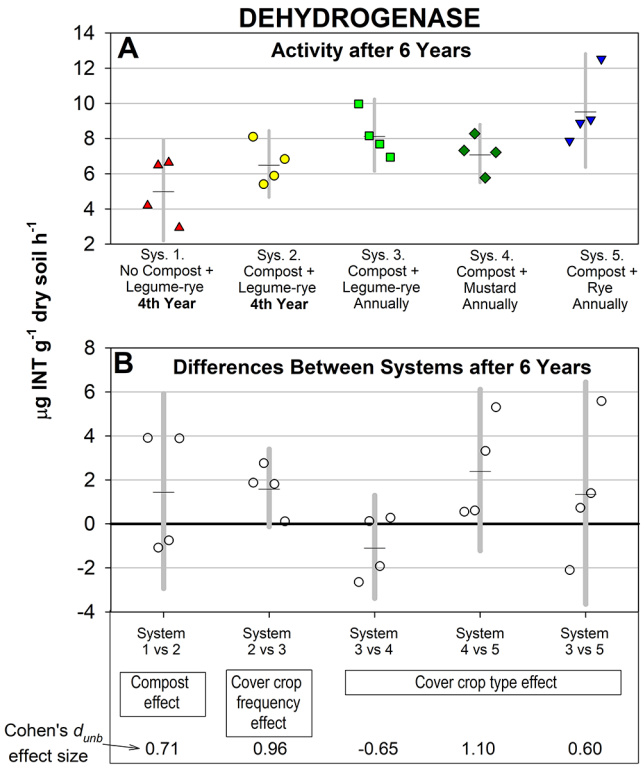

Fig. 6. Activity of Dehydrogenase after 6 yr (A) and the differences of paired comparisons between systems after 6 yr (B) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4. The standardized effect size (Cohen's unbiased *d*) is shown below each paired comparison. The boxes below the *x* axis in panel B indicate the system comparisons to evaluate the effects of the experimental factors (compost, cover crop frequency and type). See Supplemental Fig. S7A–B and S10A–C for additional details of the paired comparisons between systems.

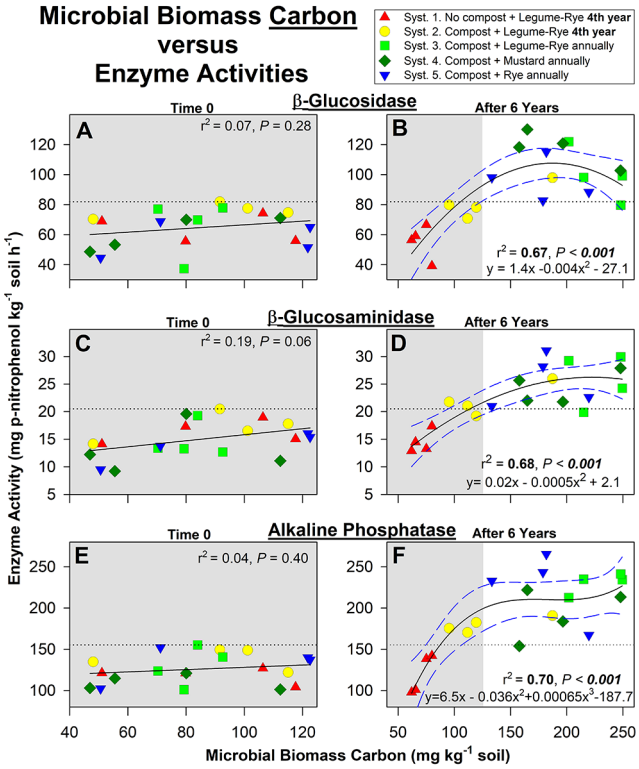

Fig. 7. Relationship between microbial biomass carbon and activities of three soil enzymes at Time 0 (A, C, E) and after 6 yr (B, D, F) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). The four colored symbols represent the four replicates for each system. The blue dashed lines in panels B, D and F are the 95% confidence bands for the fitted regression lines that were significant at $P < 0.05$; regression equations are only shown for significant relationships. Note that the scale of the *y* axes within each enzyme is the same for both times, and the scale of the *x* axes within each time is the same across all enzymes. The *x* axes for all panels begin at 40 mg C kg soil^{-1} and the gray shaded region in the panels after 6 yr illustrates the *x* axis range shown at Time 0. The horizontal dotted line in all panels indicates the highest enzyme activity at Time 0 and was 81.9, 20.5 and 155.3 mg p-nitrophenol kg soil^{-1} h^{-1} for β-glucosidase, β-glucosaminidase, and alkaline phosphatase, respectively. Additional details on microbial biomass carbon were presented by Brennan and Acosta-Martinez (2017).

As with MBC, the range of microbial biomass nitrogen (MBN) was narrower at Time 0 (4 to 15 mg N kg soil^{-1}; Fig. 8A, C, and E) than after 6 yr (6 to 40 mg N kg soil^{-1}; Fig. 8B, D, and F). Furthermore, the relationship between microbial biomass nitrogen (MBN) and enzyme activities followed the same overall pattern described above for MBC, with little evidence of a relationship between MBN and enzyme activities at Time 0, but positive relationships after 6 yr. In the fitted curves after 6 yr, the lower end included data from Systems 1 and 2 with infrequent cover cropping, while the mid to upper range of the curves was dominated by Systems 3 to 5 as occurred with MBC.

With the three enzyme activities (dehydrogenase, aspartase and L-asparaginase) evaluated only after 6 yr, there was evidence of a positive relationship for MBC and MBN with dehydrogenase and L-asparaginase (Fig. 9C–F) but not for aspartase (Fig. 9A and B). The higher r^2 values indicate that the correlations with microbial biomass were stronger for L-asparaginase than for dehydrogenase.

DISCUSSION

Soil Enzyme Research in California Vegetable and Strawberry Systems

To our knowledge our study provides the first information on how cover crops and yard-waste compost affect soil enzyme activities in high-value, tillage-intensive vegetable systems in the Salinas Valley region of California. Previous reports of soil enzymes in this region were in strawberry systems and evaluated the effect of fumigants (Klose and Ajwa, 2004; Klose et al., 2006; Stromberger et al., 2005) and organic versus conventional management (Reeve et al., 2010; Reganold et al., 2010). The fumigant studies in sandy loam soils reported β-glucosidase activities (mg p-nitrophenol kg soil^{-1} h^{-1}) that were lower (i.e., ~15 to 35, Klose and Ajwa, 2004) or similar (i.e., ~35 to 60, Stromberger et al., 2005) to the β-glucosidase activities at the start of our study (57 to 75 on average) on a sandy soil. Furthermore, the organic versus

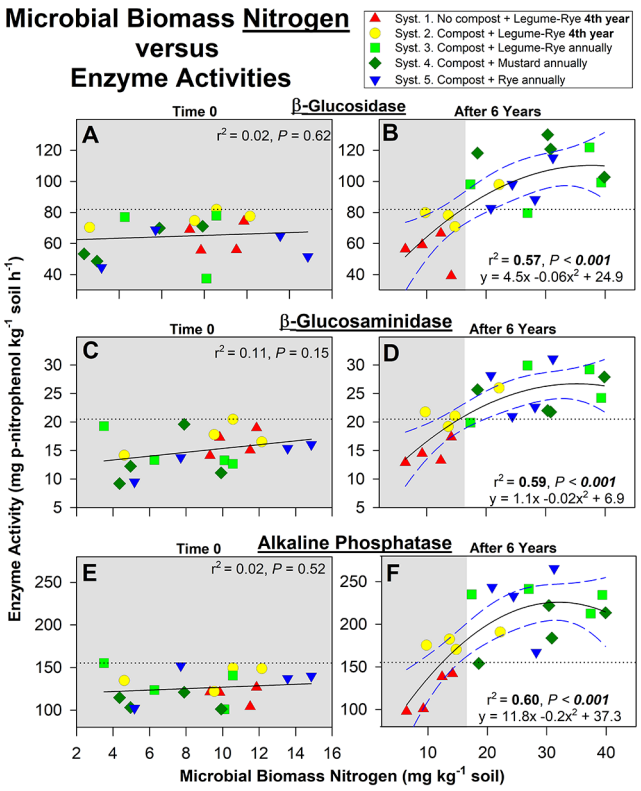

Fig. 8. Relationship between microbial biomass nitrogen and activities of three soil enzymes at Time 0 (A, C, E) and after 6 yr (B, D, F) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). The four colored symbols represent the four replicates for each system. The blue dashed lines in panels B, D and F are the 95% confidence bands for the fitted regression lines that were significant at *P* < 0.05; regression equations are only shown for significant relationships. Note that the scale of the *y* axes within each enzyme is the same for both times, and the scale of the *x* axes within each time is the same across all enzymes. The *x* axes for all panels begin at 2 mg nitrogen kg soil^{-1} and the gray shaded region in the panels after 6 yr illustrates the *x* axis range shown at Time 0. The horizontal dotted line in all panels indicates the highest enzyme activity at Time 0 and was 81.9, 20.5, and 155.3 mg p-nitrophenol kg soil^{-1} h^{-1} for β-glucosidase, β-glucosaminidase, and alkaline phosphatase, respectively. Additional details on microbial biomass nitrogen were presented by Brennan and Acosta-Martinez (2017).

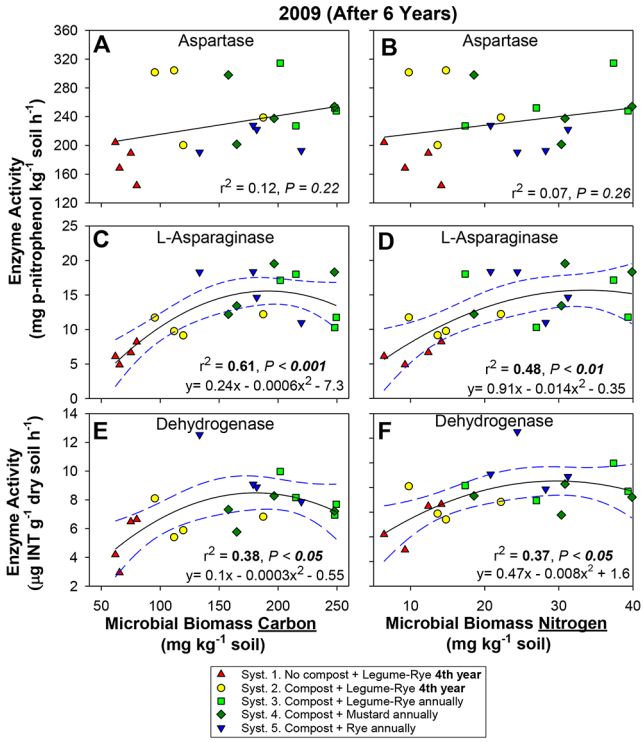

Fig. 9. Relationship between microbial biomass carbon (A, C, E) and nitrogen (B, D, F) and activities of aspartase, L-asparaginase and dehydrogenase after 6 yr in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every fourth winter). The four colored symbols represent the four replicates for each system. The blue dashed lines in panels C to F are the 95% confidence bands for the fitted regression lines that were significant at *P* < 0.05; regression equations are only shown for relationships where *P* < 0.05. Additional details on microbial biomass carbon and nitrogen were presented by Brennan and Acosta-Martinez (2017).

conventional study reported at least two-fold higher activities of dehydrogenase and alkaline phosphatase in organic fields that received approximately twice the yard-waste compost inputs (20.2 to 24.6 Mg ha^{-1}) as conventional fields (11.2 to 13.4 Mg ha^{-1}). Those compost rates were on a wet weight basis which on a dry weight basis would be approximately 60% of the reported rates because this type of compost typically has 40% moisture (Brennan, unpublished data). Therefore the highest rate would be 14.8 Mg ha^{-1} (oven-dry) which is similar to the annual rate in the four systems that received compost in our study (15.2 Mg ha^{-1}).

One of the few vegetable studies in California that measured soil enzyme activities was an observational study (Bowles et al., 2014) in clay and loam soils in organic tomatoes in Yolo county; the average daily summer temperature in Yolo county (~17 to 30°C) is typically much hotter than in Monterey county (~10 to 18°C) where our study occurred. They reported β-glucosidase activity ranging from approximately 75 to over 300 mg p-nitrophenol kg soil^{-1} h^{-1}. Cover crops were seldom used in that study, but it is interesting that within three fields in the Tahama loam soil series, β-glucosidase activity was several fold greater where vetch cover crop was the primary organic matter input than in the other fields where poultry manure was the primary organic input. This agrees with our study where β-glucosidase activity was approximately 50% greater in System 3 (cover cropped annually) than in System 1 that was infrequently cover cropped and where the primary organic matter input most years, other than from vegetable crop residue, was from pelleted organic fertilizers made from poultry manure and feather meal.

In another study in Yolo County, Geisseler and Horwath (2009) tracked changes in soil enzymes in standard versus conservation tillage in a conventionally managed tomato-corn rotation that included winter cover crops with a silty clay loam soil. The activities of β-glucosidase (174–304 mg p-nitrophenol kg soil^{-1} h^{-1}) and β-glucosaminidase (51–73 mg p-nitrophenol kg soil^{-1} h^{-1}) in both tillage treatments in that study were approximately two-fold or more higher than in the annually cover cropped systems on our study. Furthermore, β-glucosidase activity was approxi-

mately 34% higher in the conservation tilled than standard tilled system at the beginning of the corn and 21% higher at corn harvest. It is important to highlight that their 'standard' tillage practices were far less intensive (i.e., only bed-preserving disc harrowing to 15- to 20-cm depth in fall and spring) than in our study where two cash crops were grown annually with multiple tillage passes (i.e., with spading, rebedding, deep ripping, etc.). Furthermore, our systems received approximately four-fold more N per hectare annually (from organic fertilizers) than occurred during corn in the tomato-corn study.

Yard Waste Compost Effects on Enzyme Activities

The effects of compost alone on soil enzyme activities in our study can be evaluated by comparing System 1 that never received compost versus System 2 that received 91.2 Mg of compost over the 6 yr; these effects are very similar and consistent to those that were found for microbial biomass and community composition (Brennan and Acosta-Martinez, 2017) and thus will only be discussed briefly. Overall, our data indicate that yard-waste compost helped to increase the activities of most of the enzymes evaluated (Fig. 2B, 3B, 4B, and 5B). In contrast, while the effect of compost on β-glucosidase and dehydrogenase activity was positive on average, our data indicate considerable uncertainty with these enzymes (Fig. 1B and 6B). This finding is somewhat consistent with a 6 yr study on the effects of municipal solid waste compost (C to N ratio of 29.5, slightly higher than in our study) on soil enzyme activities in a sugar beet and durum wheat rotation in Spain (Crecchio et al., 2004). That study evaluated three, annual compost rates (0, 12, and 24 Mg ha^{-1}, dry weight) and reported 14 and 10% increases in activities of β-glucosidase and phosphatase after 6 yr with 12 Mg ha^{-1} but no further increase with the higher rate. In another study in Spain, inputs of 30 Mg ha^{-1} yr^{-1} of plant waste compost did not change the activity of β-glucosidase activity after 4 yr but did increase alkaline phosphatase activity compared to soil that only received mineral fertilizers (Melero et al., 2007). In contrast, in a 9-yr study in Italy, the activities of β-glucosidase, dehydrogenase and phosphatase increased with the application rate of compost made from animal manure and legume residue (Laudicina et al., 2011). It is important to note that in our study the assessment of the activities of the amidohydrolases (asparatase and L-asparaginase) and dehydrogenase between systems after 6 yr alone, are most likely less precise than with changes in the activities of the glucosidases and alkaline phosphatase where the paired differences from Time 0 to after 6 yr were measured.

Previous research has shown a negative relationship between total P availability and the activity of phosphatase enzymes (Allison et al., 2007) and that phosphatase activities and arbuscular mycorrhizae fungi (AMF) declined with P fertilizer inputs (Liu et al., 2012; Olander and Vitousek, 2000). Cumulative inputs of P over the first 8 yr of vegetable production in our study in Systems 2 through 5 receiving compost were two-fold or more greater (514 to 528 kg P ha^{-1}) than in System 1 (233 kg P ha^{-1}) without compost (Maltais-Landry et al., 2016), and resulted in higher available P (i.e., Olsen P) in the systems receiving compost. However, contrary to the studies noted above, phosphatase activity was not negatively correlated with P availability. Our findings are consistent with other studies in California that showed that higher rates of compost in organic strawberry systems resulted in higher phosphatase activities than occurred in conventional fields receiving less compost, but that available P levels were similar between systems (Reganold et al., 2010). Similarly, in organic tomato systems in California, Bowles et al. (2014) did not find an association between P availability and phosphatase activity. Long-term data from the present study and another one in California showed that P inputs in organic systems often far exceed P exports in harvested product (Maltais-Landry et al., 2015, 2016). Excessive P inputs and intensive tillage in all systems in the present study may explain why AMF declined in all systems and was unaffected by management differences (Brennan and Acosta-Martinez, 2017, see for additional discussion of AMF in this study).

Cover Crop Effects on Enzyme Activities

The scientific literature from experimental studies (i.e., excluding observation studies) on the effects of cover crops on soil enzymes in vegetable systems is extremely limited. For example, our recent search of the Web of Science database for the topic words 'cover crop', 'enzyme' and 'vegetable' resulted in only six, relevant citations (Bandick and Dick, 1999; Hamido and Kpomblekou-A, 2009; Mancinelli et al., 2013; Mendes et al., 1999; Pritchett et al., 2011; Tian et al., 2013). While this may not represent all of the published work on these topics, it provides strong evidence of the need for more research in this area. More research is needed to improve our understanding of the mechanisms by which cover crops influence soil enzyme activities and other soil attributes, and this will require basic information on cover crop biomass production and quality. Unfortunately, such information was often not reported in past studies on cover crop effects on soil enzymes which limits their value; this omission includes some of the most widely cited papers on the effects of agricultural management on soil enzymes. Cover crop biomass inputs and quality essentially represent the amount of 'active ingredient' that a cover crop adds to a system and therefore should always be reported when cover crops are evaluated. This information is critical to understand the effects of cover crops versus fallow, the effects of cover crop type, and comparisons of cover crops with other imported organic matter amendments such as the yard-waste compost used in our study. Long-term studies on cover crop effects on soil enzymes in Sweden (Elfstrand et al., 2007) and Brazil (Balota et al., 2014) are excellent examples where detailed information of organic matter inputs from cover crops were provided. Both of these studies highlighted the potential impact of organic matter inputs and quality on soil enzyme activities. We draw attention to this issue to encourage researchers to consistently report cover crop biomass production in future reports on cover crop effects on soil quality or health. Interested readers can obtain detailed information on cover crop shoot biomass inputs, residue quality, and nitrogen content in the present long-term study from these publications (Brennan and Boyd, 2012a, 2012b; Brennan and Smith, 2017; Brennan et al., 2013).

Several studies illustrate the complex and somewhat inconsistent effects of plant residues on soil enzyme activities that seem relevant to our study. For example with different legume cover crops, Dinesh et al. (1999) found that the activities of several soil enzymes were positively correlated with the amount of cover crop shoot biomass that was added. Whereas, in a study with peppers, Mancinelli et al. (2013) found that even with similar cover crop biomass inputs, soil enzyme activities were higher on average following vetch than ryegrass cover crops during the second year of the study. Furthermore, a study that added the same amount of organic C from shoot residue from crops in several plant families found considerable variability in soil enzymes activities even within residue from the same family (Perucci et al., 1984). In our study, mustard was typically less productive than rye and the legume-rye mixture (Brennan and Boyd, 2012a) such that cumulative mustard shoot inputs over the first 6 yr were considerably lower (30.9 Mg ha^{-1}, System 4) than for rye (43.1 Mg ha^{-1}, System 5) or the legume-rye mixture (45.6 Mg ha^{-1}, System 3) (Table 1). Despite these differences in season-end cover crop shoot inputs, shoot residue quality (C to N ratio: mustard = 22, legume-rye = 21, rye = 29, Brennan et al., 2013), and N content (kg ha^{-1}: mustard = 114, rye = 110, legume-rye = 151, Brennan and Boyd, 2012b), our study indicates that these differences had relatively little if any effect on soil enzyme activities. Overall our data showing similarities in soil enzymes across all cover crop types are consistent with the similarities between these systems in microbial biomass C and N (Brennan and Acosta-Martinez, 2017).

Relevance of this Research to the USDA National Organic Standards

The Soil Fertility and Crop Nutrient Management Practice Standard of the USDA National Organic Program states that 'the producer must select and implement tillage and cultivation practices that maintain or improve the physical, chemical, and biological condition of soil and minimize soil erosion.' The soil enzyme data from our study combined with other measures of soil biology in this experiment (i.e., nematode community analysis, Ferris et al., 2012; microbial community size and composition, Brennan and Acosta-Martinez, 2017), provide compelling evidence that the infrequently cover cropped systems were degrading soil biological conditions (System 1, no compost) or providing relatively little improvements (System 2, with compost) compared with the clear improvements that occurred in the annually cover cropped systems (System 3, 4, and 5). This highlights the critical value of studies within organic systems; unfortunately, such work is extremely uncommon; what is far more common are studies that compare organic versus conventional systems. These three lines of evidence (i.e., nematodes, microbial biomass, and soil enzymes) from this systems experiment raise questions about whether the current USDA organic regulations are adequate to foster and ensure best soil management practices in high-input organic systems. It also highlights the importance of developing novel approaches that enable and encourage farmers to integrate cover crops regularly into vegetable systems (Brennan, 2017a, 2017b).

Practical Implications for Soil Health

The five certified organic systems described in this paper differed considerable in the amount of organic matter they received from cover crop shoots and compost over the 6 yr with System 2 receiving 13.4-fold more than System 1, and Systems 3, 4, and 5 receiving 18.5-, 16.5- and to 18.1-fold more than System 1, respectively (see Fig. 1 in Brennan and Acosta-Martinez, 2017; Table 1). This resulted in differences between many of these systems in soil enzyme activities (e.g., Fig. 1 to 3), and microbial biomass and community composition (Brennan and Acosta-Martinez, 2017), and showed that the greatest changes in most enzyme activities over time occurred in the three systems that received compost and cover crops annually. These annually cover cropped systems (System 3, 4, 5) also produced higher and less variable yields of broccoli and lettuce over the first 8 yr of this study than were produced in the infrequently cover cropped systems (see Table 1 in Maltais-Landry et al., 2015; additional details of crop yields will be reported in future publications from this study). As with several previous studies in a variety of systems and soil types (Dick et al., 1988; Dodor and Tabatabai, 2003b; Ekenler and Tabatabai, 2002; Frankenberger and Dick, 1983; Herencia, 2015) our results show that enzyme activities were correlated with microbial biomass C and N.

Although our study lacked systems with annual cover cropping without compost, we speculate that the C inputs from frequent cover cropping had a greater proportional influence than compost on soil enzyme activities. This reasoning is supported by the relatively small average increase in β-glucosidase activity that occurred after 6 yr in System 2 (6 [CI = −18, 28] mg p-nitrophenol kg soil^{-1} h^{-1}) compared with System 3 (34 [−2, 71] mg p-nitrophenol kg soil^{-1} h^{-1}), both which received 91.2 Mg ha^{-1} of compost over the 6 yr (Fig. 1B; Supplemental Fig. S2 panels B and C). It is interesting to note that the 5.7-fold greater average increase in β-glucosidase activity after 6 yr that occurred in System 3 than System 2 (i.e., 34 ÷ 6 = 5.7) is equivalent to the 5.7-fold more organic matter input from cover crop shoots in System 3 (45.6 Mg ha^{-1}) than System 2 (8 Mg ha^{-1}) during that time (Table 1). While this suggests that the changes in β-glucosidase activity were essentially proportional to the cover crop shoot inputs, research by Martens et al. (1992) that tracked β-glucosidase activities several times annually over 3 yr following repeated and large inputs of organic amendments (i.e., 25 Mg dry matter ha^{-1}), showed that increases were greatest after the first addition, were not additive, and were greater with additions of cereal straw and alfalfa than from manure or sludge. This finding agrees somewhat with our study that found that inputs of organic matter from fresh plant tissue from cover crops often had a greater impact on enzyme activities than occurred with compost and infrequent inputs of cover crop; however, aspartase activity did not appear to be affected by annual C inputs from cover crops (Fig. 4B).

Beta-glucosidase is an important enzyme in C cycling in the soil and has been suggested as a sensitive indicator of soil quality changes (Bandick and Dick, 1999; Stott et al., 2010). In our study, β-glucosidase showed differences between systems (Fig. 1)

and thus appears to be a reliable indicator of soil health changes in these tillage-intensive vegetable systems. This is consistent with the increases in β-glucosidase activity in a vegetable rotation that occurred with cover cropping despite no change in soil organic carbon (Dick, 1994). Pritchett et al. (2011) similarly found that β-glucosidase activity differed with cover cropping while dehydrogenase activity did not. The changes in the activity of β-glucosidase in our vegetable systems may have been caused by microbial production in the soil or from inputs of cover crop or compost with high enzyme activities. We are not aware of any reports of enzyme activities in yard-waste compost like what we used, but Martens et al. (1992) reported 70-fold or more greater activities of β-glucosidase in straw and alfalfa than in poultry manure and sewage sludge. In tillage-intensive, vegetable production in plastic tunnels with various compost rates, the activity of β-glucosidase was positively correlated with vegetable yields (Bonanomi et al., 2014). Furthermore, in an observational study comparing organic versus conventional vegetable farms in the tropics, Moeskops et al. (2012) reported higher β-glucosidase activity in soil from some organic than conventional sites. However, as our experimental study within organic systems illustrates clearly, soil enzyme activities are related to specific management practices (i.e., cover cropping frequency and compost inputs). While microbial biomass and β-glucosidase activity were clearly linked to management differences between systems in our study that occurred on a laser-leveled field with a uniform slope of approximately 1%, the effects of management on this enzyme may be more complex in regions with more topological diversity (Wickings et al., 2016).

There is relatively little published information on the soil management effects of aspartase activity, however, long-term research in Iowa found that the activity of this enzyme was correlated significantly with MBN and MBC and that it was also affected by cropping system and N fertilization (Dodor and Tabatabai, 2003a). This contrasts with our study where there was no apparent correlation between aspartase activity and MBC or MBN (Fig. 9A and B). Furthermore, there was no evidence in our study that the increased cover cropping intensity in System 3 than System 2 had any impact on this enzyme activity (Fig. 4B). In our study, compost was the only input that appears to have affected aspartase activity. It is possible that the high level of tillage in our study explain why our results do not agree with previous work on this enzyme. Research in Wisconsin showed that aspartase activity was sensitive to increased tillage intensity (Senwo and Tabatabai, 2005).

CONCLUSIONS AND FUTURE RESEARCH NEEDS

This study provides important information on soil enzyme activity in a loamy sand soil in the Salinas Valley and illustrates how this was affected by organic matter inputs from yard waste compost and winter cover cropping in high-input, tillage-intensive, organic vegetable systems. Combined with previous analyses of other sensitive indicators of soil health in this long-term study (i.e., nematodes, Ferris et al., 2012; microbial biomass, Brennan and Acosta-Martinez, 2017) the present paper provides additional evidence of the benefits of annual winter cover cropping in intensive vegetable systems. In contrast, there were relatively small improvements in enzyme activities that occurred in the system with annual inputs of yard-waste compost and infrequent cover cropping. Our data indicate that in the annually cover cropped systems, differences in the quantity, quality, and type of cover crop biomass had little if any influence on soil enzyme activities involved in biogeochemical cycling. While yard-waste compost is a more convenient way to add organic matter to the soil and increased soil organic carbon over time (Brennan and Smith, 2017; Brennan and Acosta-Martinez, 2017), analysis of P budgets from the study (Maltais-Landry et al., 2016) indicated that compost also contributes to excessive P inputs. This highlights the problem in vegetable systems with the over reliance on compost from off-farm sources. We therefore suggest that future studies on management effects in high-input, tillage intensive vegetable systems in this region and elsewhere include treatments with annual cover cropping without compost. It would also be helpful to evaluate changes over shorter increments (i.e., annually, and several times within a year) than occurred in the present study and evaluate changes in clay and loam soils. Overall, our analysis of soil enzyme activities agrees with previous reports on soil health changes in this long-term experiment (Brennan and Acosta-Martinez, 2017; Ferris et al., 2012) and illustrates that these changes are caused by specific management practices such as cover cropping that can differ markedly within organic systems. To provide insights into the mechanisms that enable cover crops to improve soil health, we highlight the need for researchers to always report basic information on the quantity and quality of cover crop biomass added to the soil. This basic information will help us to understand the complex effects of cover crops and will provide more useful data for future meta-analyses. We hope that the results presented in this and our other soil health related papers from this long-term study lead to the more frequent use of cover crops in organic and conventional systems in regions like the Salinas Valley of California. The growing body of information from this important long-term trial challenges the overly simplistic and misleading notion that certified organic management improves soil health or quality, and raises concerns about the adequacy of the existing USDA organic regulations to foster best soil management practices in high-input organic systems.

SUPPLEMENTAL MATERIAL

Supplemental materials is available with the online version of this article. The first supplemental document is Supplemental Fig. S1 to help readers visualize CIs in the shape of a 'cat's eye'. The second contains Supplemental Fig. S2 through S10 that show additional information from ESCI of the paired comparisons of interest. See the text on statistical analysis above for more details on the potential value of this material.

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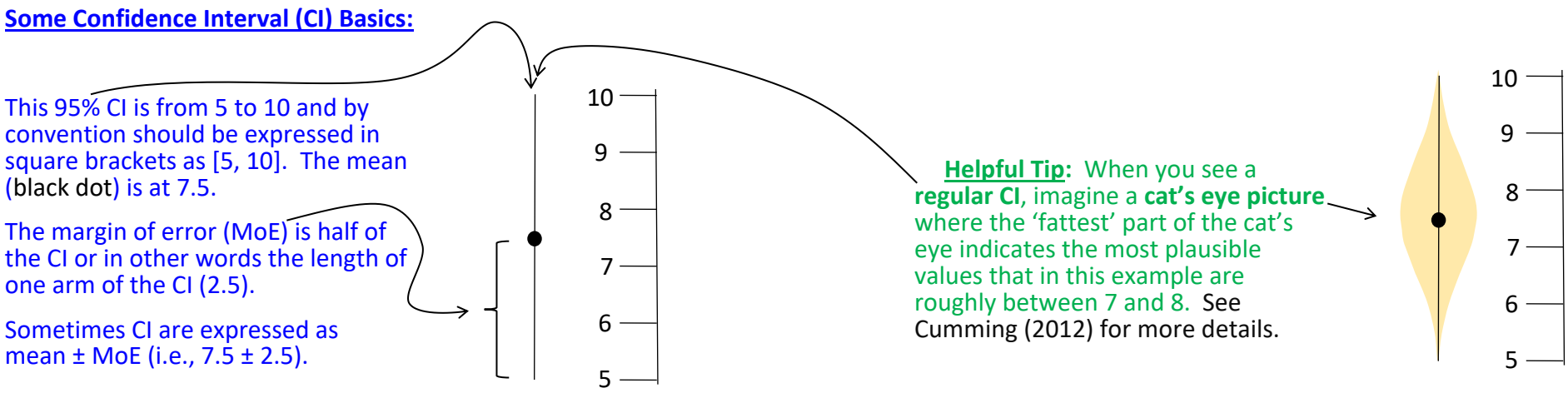
Supplemental Figure 1.



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Supplemental Figures 2 to 10 (Summary sheet & clickable Table of Contents)

Analyses to Help Readers Understand & Intreprete Confidence Intervals (CIs)

The following 13 pages have screen shots (in blue boxes) from the analysis of enzyme activities within the five Systems in the SOCS experiment that were done in the **Exploratory Software for Confidence Intervals** (ESCI). ESCI is a set of files for MS Excel developed by Geoff Cumming that is freely available from this site and designed to accompany these books (Introduction to the New Statistics and Understanding the New Statistics). In ESCI, the *Data Paired* sheet was used for within system comparisons for changes from Time 0 to Year 6 for analysis β-Glucosidase, β-Glucosaminidase and Alkaline phosphatase. The *Data Paired* sheet was also used for paired comparison among the legume-rye systems (Systems 1, 2, 3) to evaluate compost and cover crop frequeny effects, and for paired comparisons among the annually cover cropped systems (Systems 3, 4, and 5) to evaluate cover crop type effects. See table 1 in the paper for more details in these comparisons.

The **raw data** for the 5 systems of focus in the paper and for the additional 3 systems (with annual cover crops at other seeding rates) are available in Brennan and Acosta-Martinez (2018. Soil microbial biomass and soil enzymes data after six years of cover crop and compost treatments in organic vegetable production. Data in Brief 21:212-217). The raw data are provided to give interested readers a chance to use it in ESCI, and to facilitate future meta-analyses.

Hopefully this information will help readers to (1) become more familiar and confident ☺ with using CIs to make statistical inferences for paired data, and (2) understand how CIs relate to p-values from null hypothesis significance tests.

-Below is a **clickable Table of Contents** to help you navigate. You can return to this page by clicking *Return to Table of Contents* in the pages below.

Analyses of β-Glucosidase, β-Glucosaminidase and Alkaline phosphatase to compare:

Analyses of Aspartase, L-Asparaginase and Dehydrogenase at year 6 alone to compare:

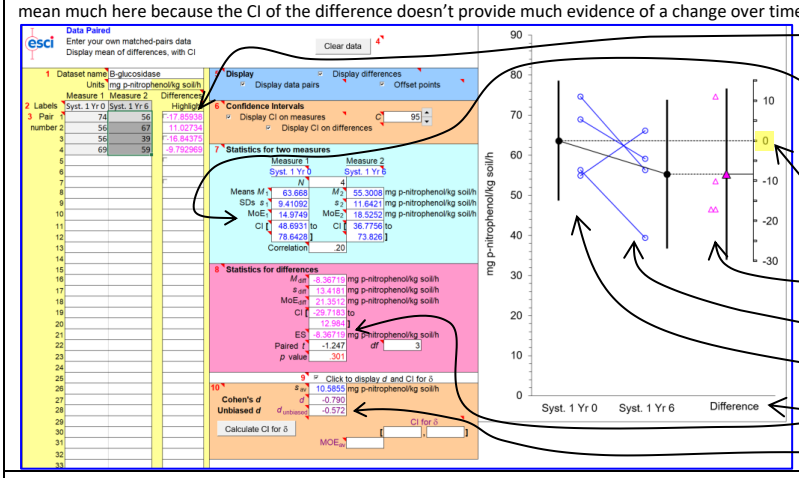
* Note that the screen shots of the analysis of β-Glucosidase activity have detailed summaries including some cat's eye pictures. But for the other enzymes only screen shots are provided.

Supplemental Figure 2 (A-E)

Comparison of Changes in β-GLUCOSIDASE activity within Systems from Time 0 to After 6 years

2A. System 1 (No compost + Legume-rye 4th year) Time 0 versus After 6 Years.

The figure shows the mean for the system at the two times with the raw data points as blue circles. The blue lines connecting the circles help remind us that this is paired data and show the direction of the change (a decline in 3 replicates and an increase in one). The size of the change in each pair is shown in pink in the differences column, and as pink triangles in the figure. The large amount of overlap in the confidence interval (CI) of the paired difference [-30, 13] with zero agrees with the p-value (0.301) for the test of the null hypothesis that there is no difference in the change in enzyme activity from time 0 and after 6 years. This provides evidence of no change in the activity of β-glucosidase over the 6 years in System 1. Note that we can see this conclusion just by looking at the CI of the difference. We can also see that one pair (replicate 2) that increased is having a large influence on the width of the CI. The effect size measure (Cohen's unbiased *d*, d_{unb}) doesn't mean much here because the CI of the difference doesn't provide much evidence of a change over time, but in some of the examples below it will be important.



Summary

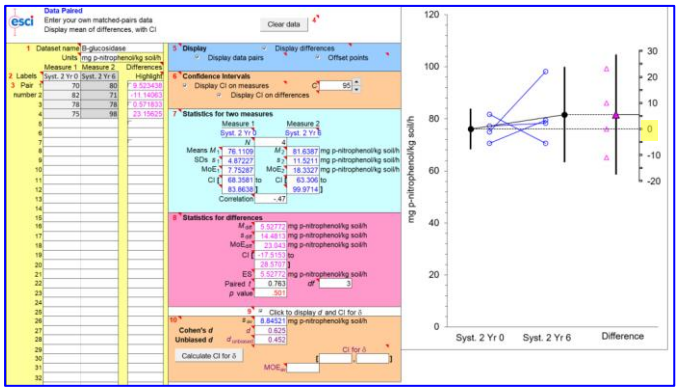
-Mean difference: **-8 [-30, 13]**

-3 of the 4 replicates decreased & 1 increased

-**p-value=0.301** of testing the null hypothesis that here is no difference from time 0 and 6 years

-**little evidence of a change** in enzyme activity, but the wide CI shows lots of variability.

2B. System 2 (Compost + Legume-rye 4th year) Time 0 versus After 6 Years.



Summary

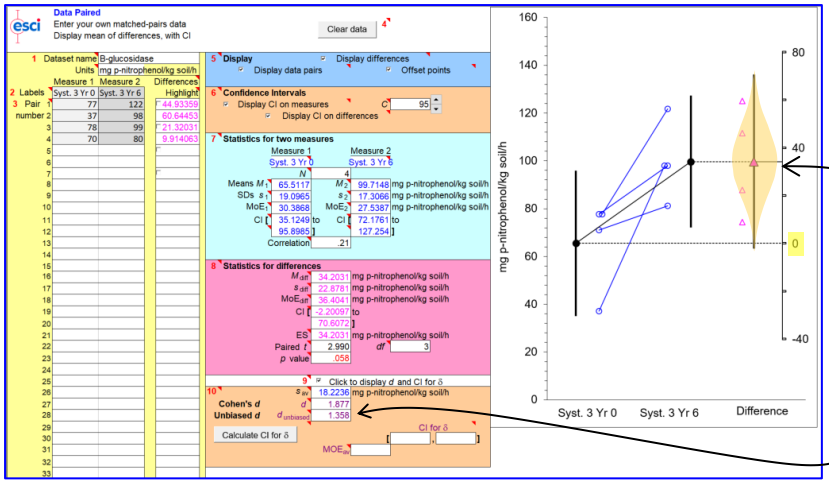
-Mean difference: **6 [-18, 29]**

-2 of the 4 replicates increased, 1 decreased and 1 had little change

-**p-value=0.501** of testing the null hypothesis that here is no difference from time 0 and 6 years

-**little evidence of a change** in enzyme activity, but the wide CI shows lots of variability.

2C. System 3 (Compost + Legume-rye 4th annually) Time 0 versus After 6 Years.



Summary

- Mean difference: **34 [-2, 71]**

-all 4 replicates increased but the increase varied from 10 to 61 units.

-**p-value= 0.058** of testing the null hypothesis that here is no difference from time 0 and 6 years. The p-value is slightly larger than 0.05 because the lower limit of the CI overlaps slightly with 0.

-**evidence that enzyme activity increased** over time despite the variability in the data as indicated by the wide CI of the difference. Keep in mind the **cat's eye picture** (shaded light brown) that suggests that the increase in enzyme activity is most plausible around 30 to 40 units where the **cat's eye** is fattest. This is the only legume-rye system where there was a consistent increase in enzyme activity over time.

-d_{unb}**=1.36** is relatively large.

(See next page for comparison within Systems 4 and 5)

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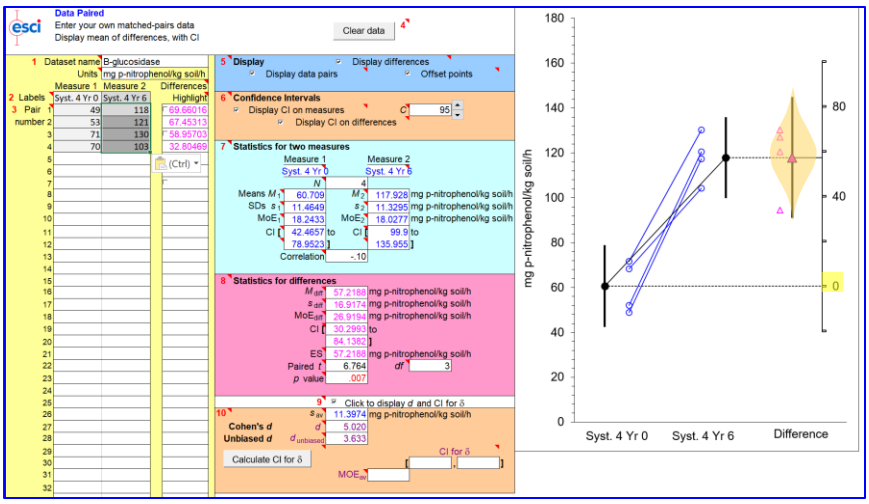
(Continued)

2D. System 4 (Compost + Mustard annually) Time 0 versus After 6 Years.



Summary

-Mean difference: **57 [30, 84]**

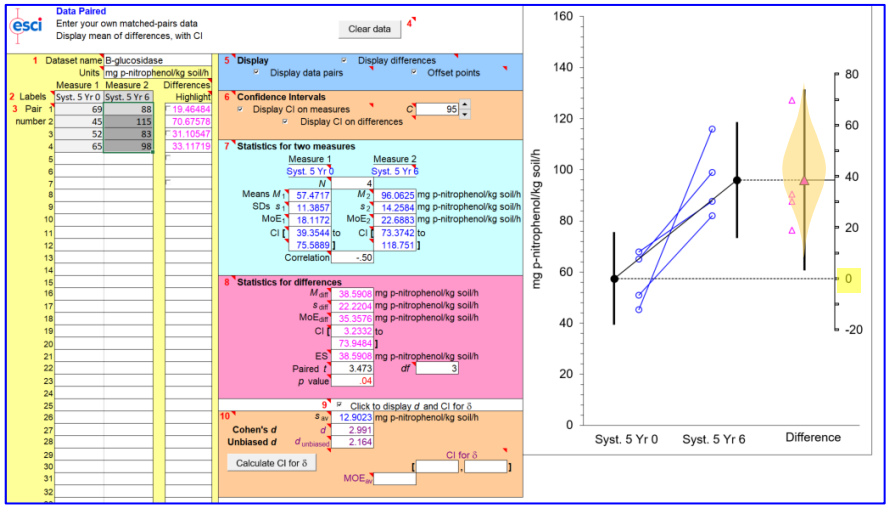
-all 4 replicates increased with 3 increasing more than 59 units and 1 increasing by 33 units

-**p-value=0.007** of testing the null hypothesis that here is no difference from time 0 and 6 years.

-**strong evidence** that enzyme activity increased over time by about 50 to 60 units.

-d_{unb}**=3.63** is large which agrees with the large increase in enzyme activity over time. It makes sense that d_{unb} in System 4 is larger than in Systems 3 and 5, because in System 4 the mean difference is greater and the CI is narrower than in Systems 3 and 5.

2E. System 5 (Compost + Rye annually) Time 0 versus After 6 Years.



Summary

-Mean difference: **39 [3, 74]**

-all 4 replicates increased with 3 increasing by 19 to 33 units, and 1 increasing by 71 units

-**p-value=0.04** of testing the null hypothesis that here is no difference from time 0 and 6 years. Note that the p-value is slightly less than 0.05 because the lower limit of the CI is relatively close to 0.

-**evidence that enzyme activity** increased over time by about 30 to 50 units

-d_{unb}**=2.16** is large which agrees with the large average change in enzyme activity.

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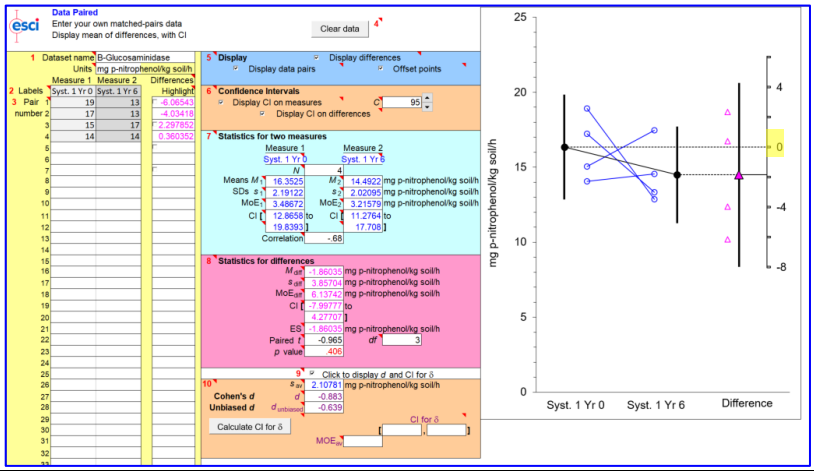
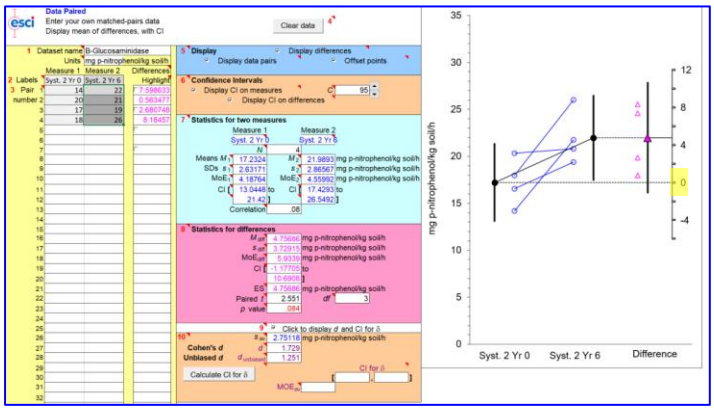
Supplemental Figure 3 (A-E)

Comparison of Changes in β-GLUCOSAMINIDASE activity within Systems from Time 0 to After 6 years

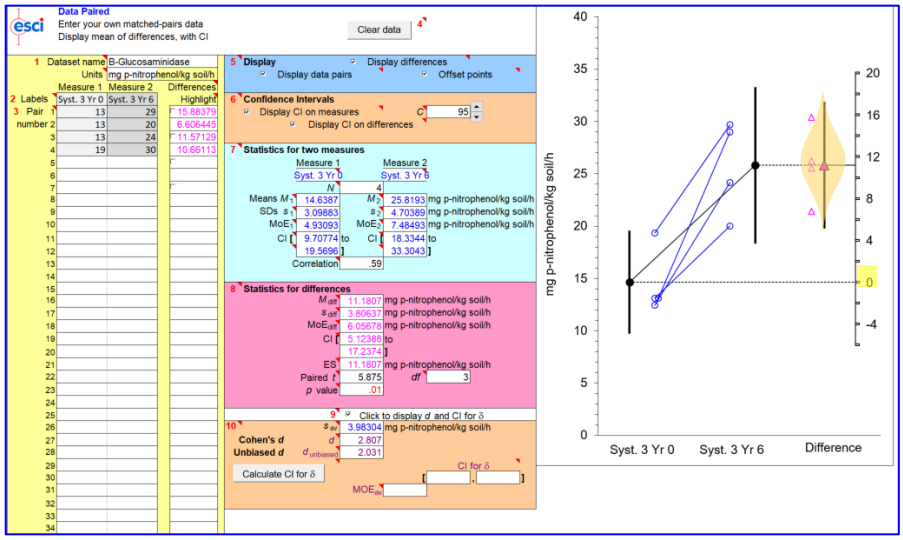
3A. System 1 (No compost + Legume-rye 4th year) Time 0 versus After 6 Years.



3B. System 2 (Compost + Legume-rye 4th year) Time 0 versus After 6 Years.



3C. System 3 (Compost + Legume-rye 4th annually) Time 0 versus After 6 Years.



(See next page for comparison within Systems 4 and 5)

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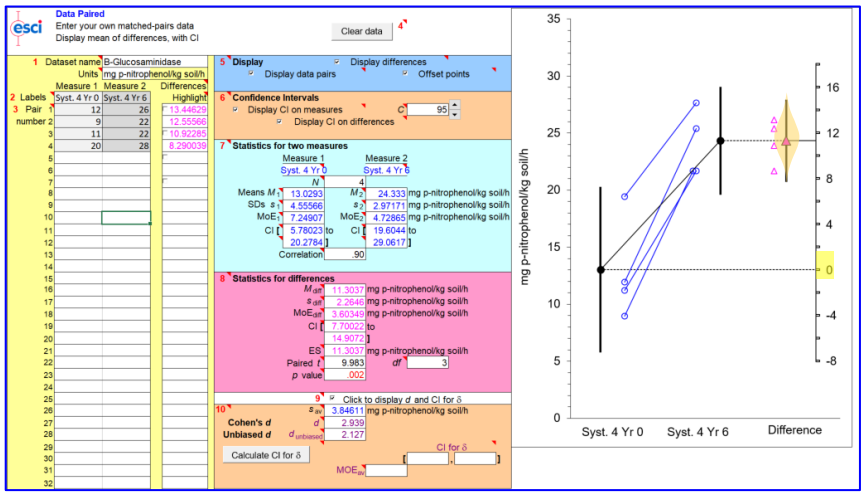
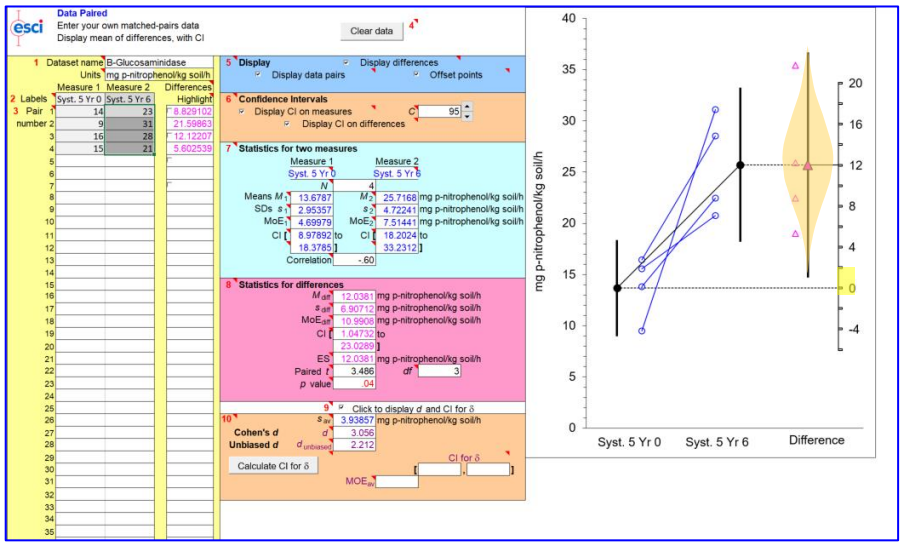
(continued)

3D. System 4 (Compost + Mustard annually) Time 0 versus After 6 Years.



3E. System 5 (Compost + Rye annually) Time 0 versus After 6 Years.



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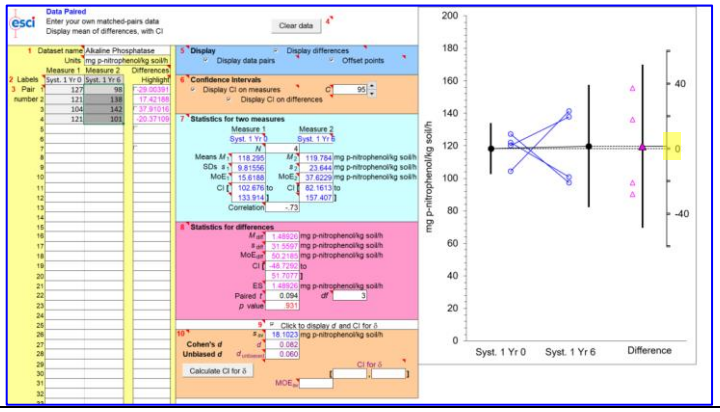
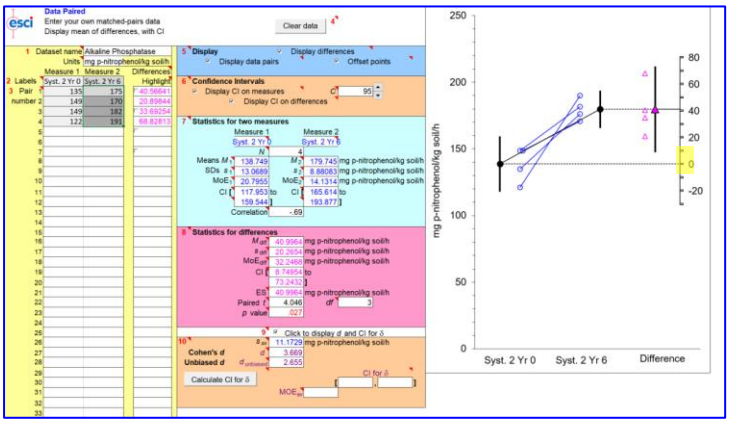
Supplemental Figure 4 (A-E)

Comparison of Changes in ALKALINE PHOSPHATASE activity within Systems from Time 0 to After 6 years

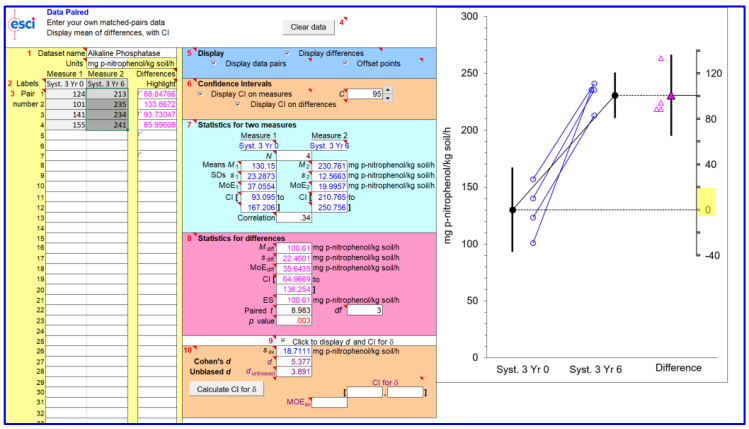
4A. System 1 (No compost + Legume-rye 4th year) Time 0 versus After 6 Years.



4B. System 2 (Compost + Legume-rye 4th year) Time 0 versus After 6 Years.



4C. System 3 (Compost + Legume-rye 4th annually) Time 0 versus After 6 Years.



(See next page for comparison within Systems 4 and 5)

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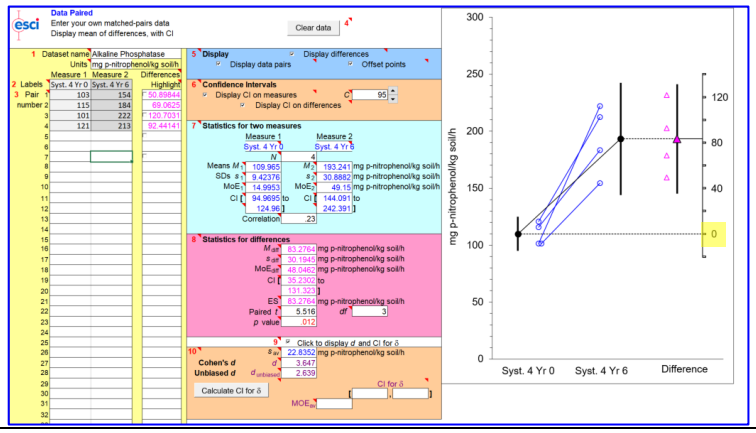
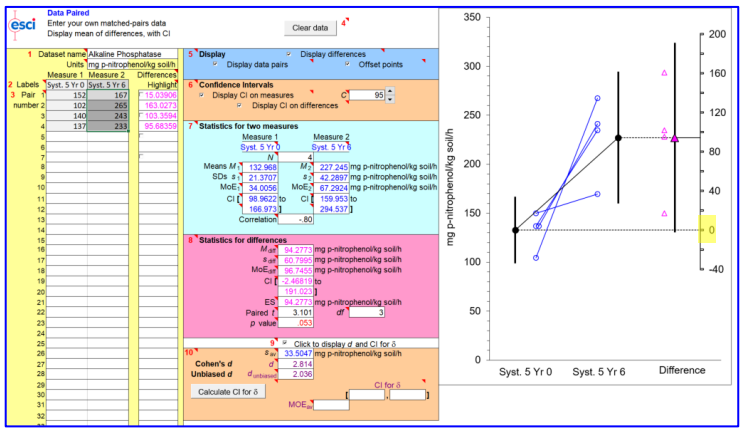
(continued)

4D. System 4 (Compost + Mustard annually) Time 0 versus After 6 Years.



4E. System 5 (Compost + Rye annually) Time 0 versus After 6 Years.



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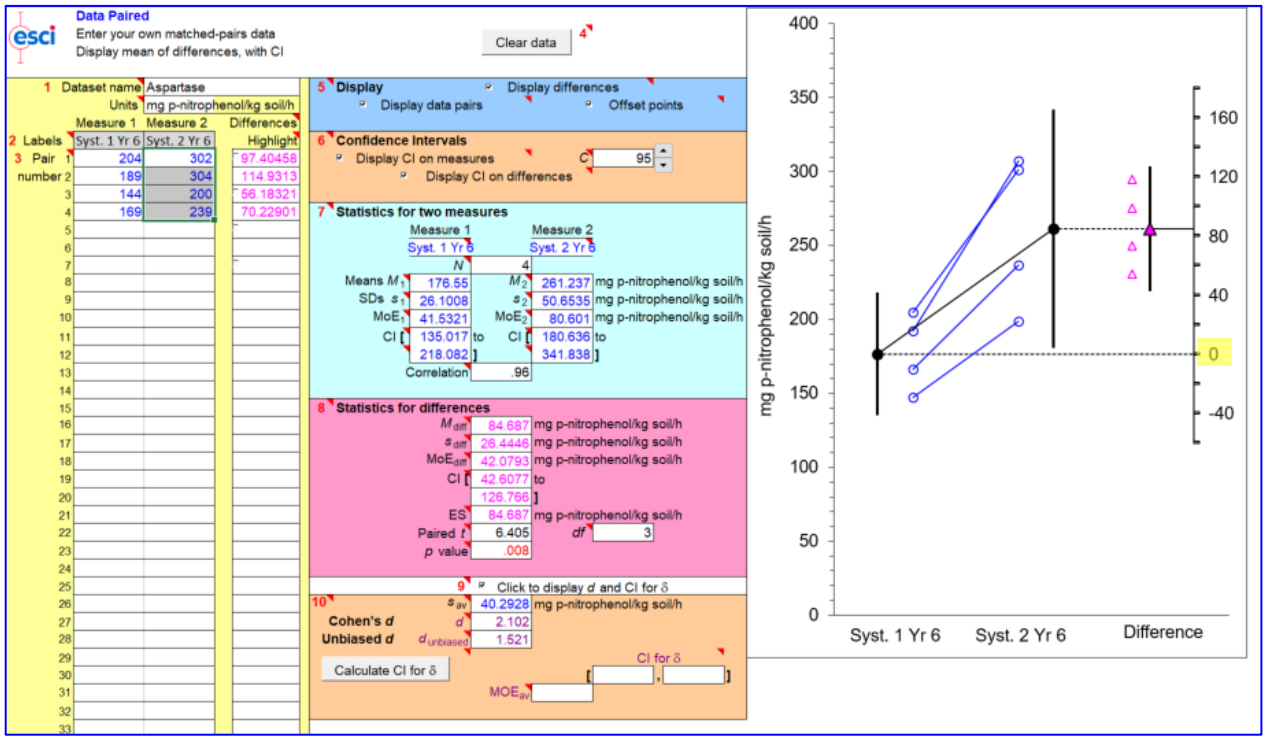
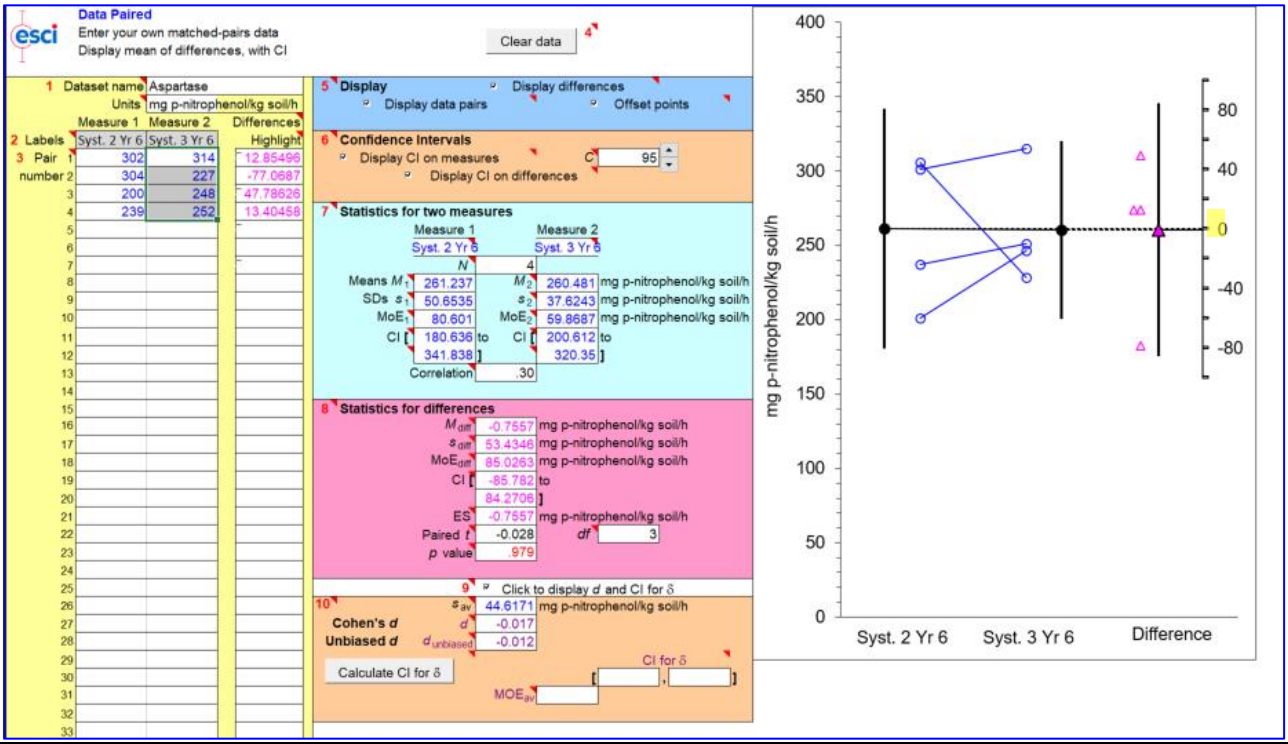
Supplemental Figure 5 (A-B)

Comparison of ASPARTASE activity between Legume-Rye Systems 1, 2 & 3 that differed in Compost and Cover crop after 6 years

5A. System 1 (No compost + Legume-rye 4th year) versus System 2 (Compost + Legume-rye 4th year).



5B. System 2 (Compost + Legume-rye 4th year) versus System 3 (Compost + Legume-rye annually)



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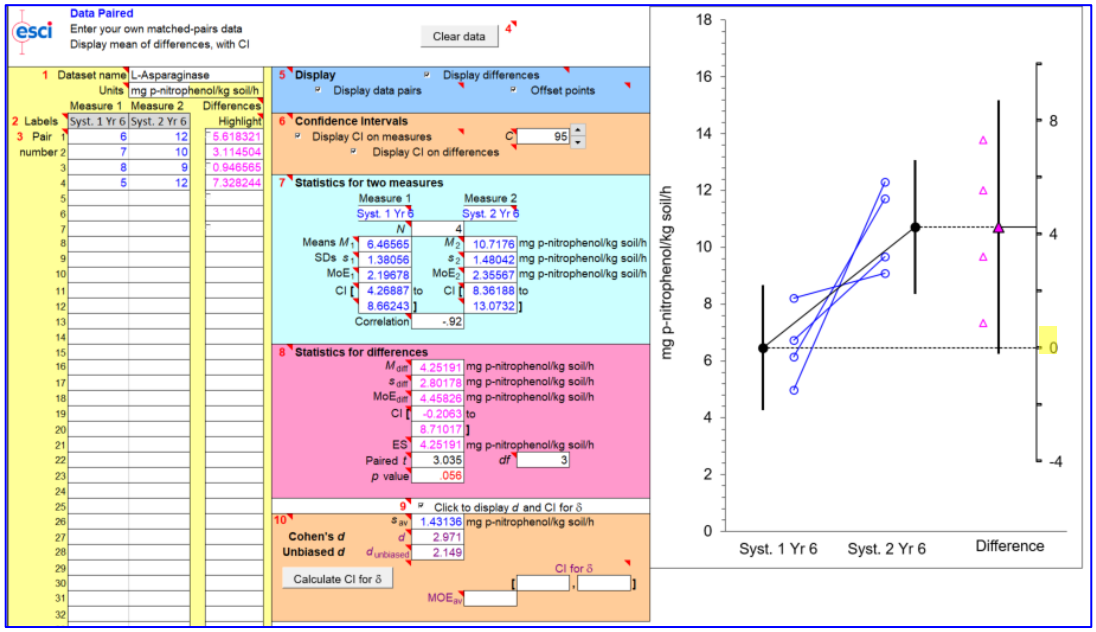
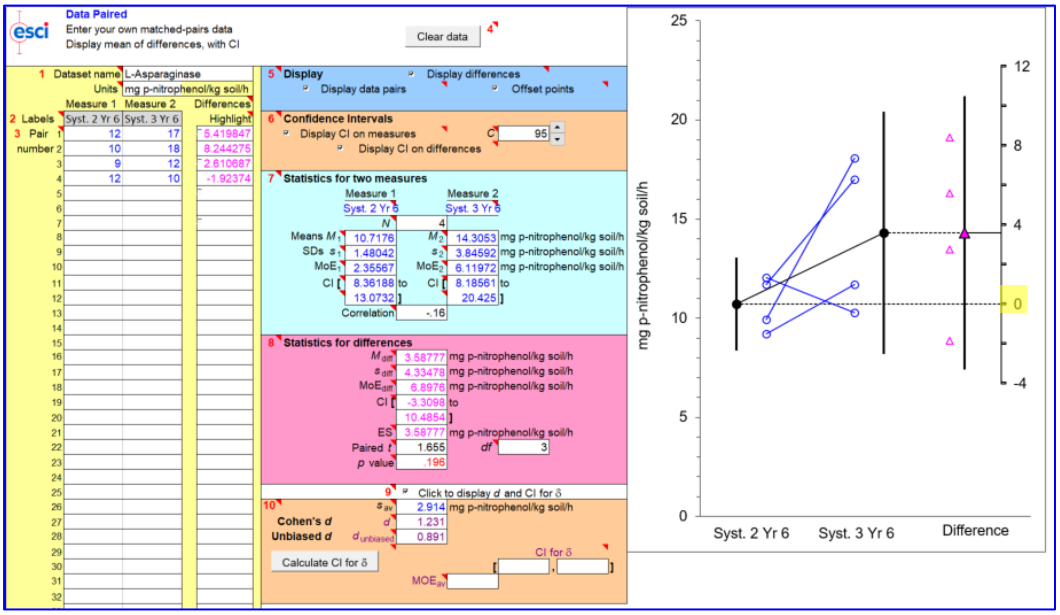
Supplemental Figure 6 (A-B)

Comparison of L-ASPARAGINASE activity between Legume-Rye Systems 1, 2 & 3 that differed in Compost and Cover crop after 6 years

6A. System 1 (No compost + Legume-rye 4th year) versus System 2 (Compost + Legume-rye 4th year).



6B. System 2 (Compost + Legume-rye 4th year) versus System 3 (Compost + Legume-rye annually)



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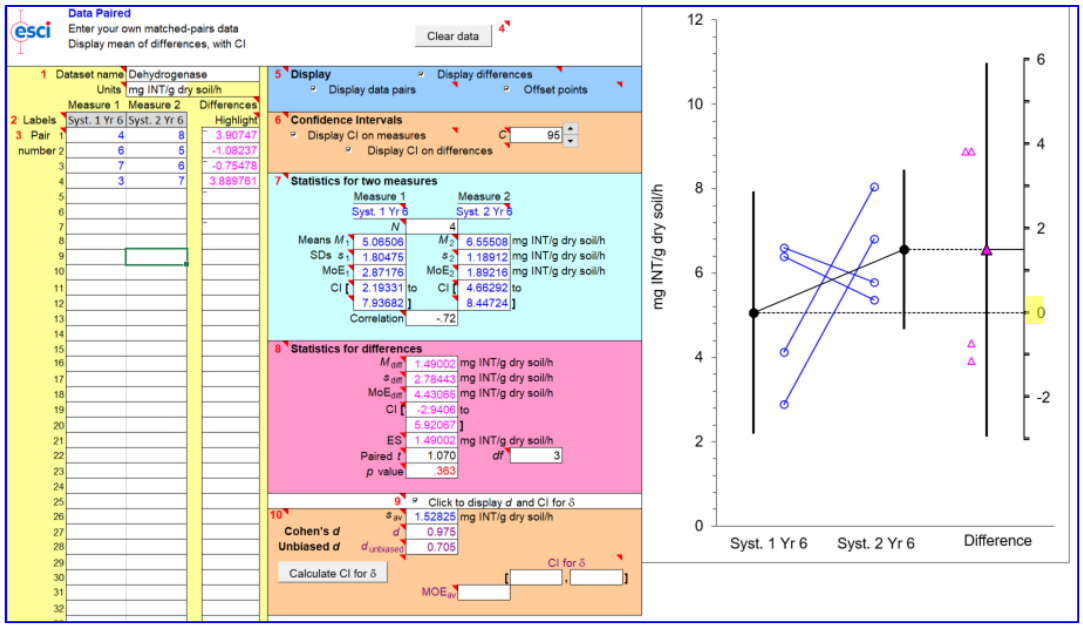
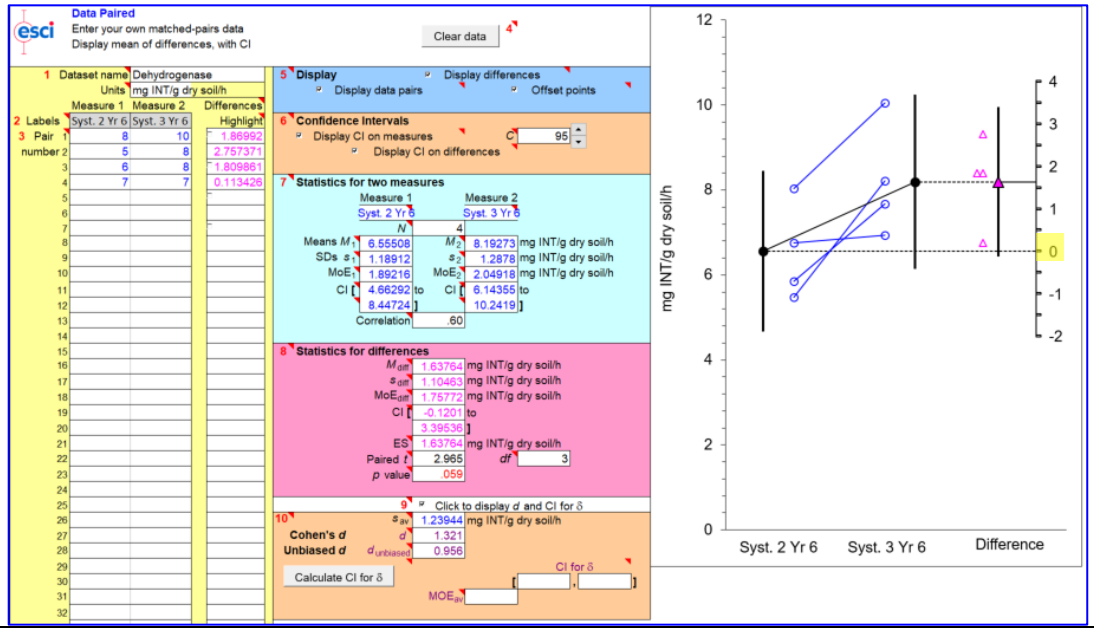
Supplemental Figure 7 (A-B)

Comparison of DEHYDROGENASE activity between Legume-Rye Systems 1, 2 & 3 that differed in Compost and Cover crop after 6 years

7A. System 1 (No compost + Legume-rye 4th year) versus System 2 (Compost + Legume-rye 4th year).



7B. System 2 (Compost + 4th year) versus System 3 (Compost + Legume-rye annually)



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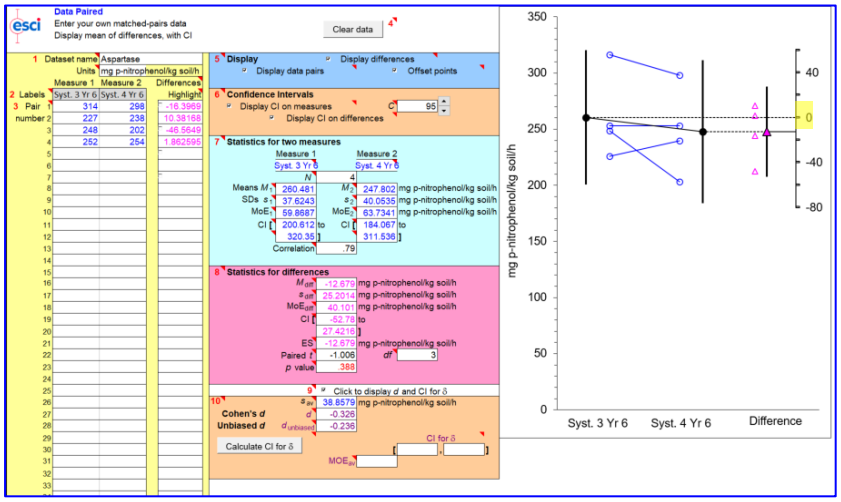
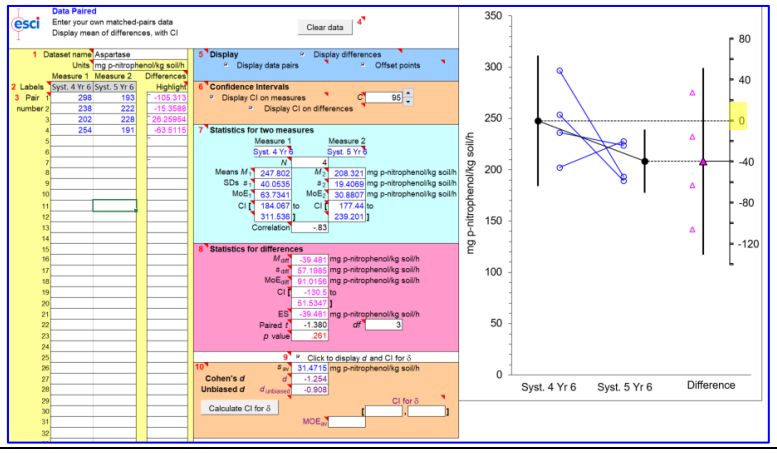
Supplemental Figure 8 (A-C)

Comparison of ASPARTASE activity between the three Cover Crop types After 6 years

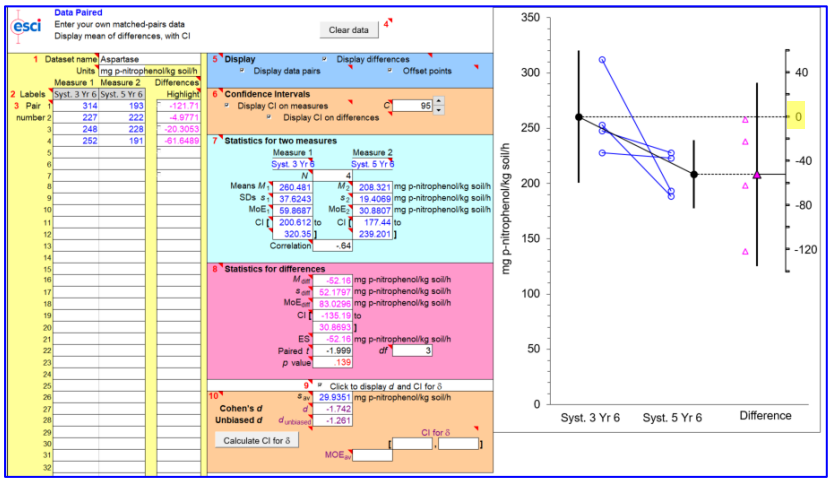
8A. System 3 (Compost + Legume-rye annually) versus System 4 (Compost + Mustard annually)



8B. System 4 (Compost + Mustard annually) versus System 5 (Compost + Rye annually)



8C. System 3 (Compost + Legume-rye annually) versus System 5 (Compost + Rye annually)



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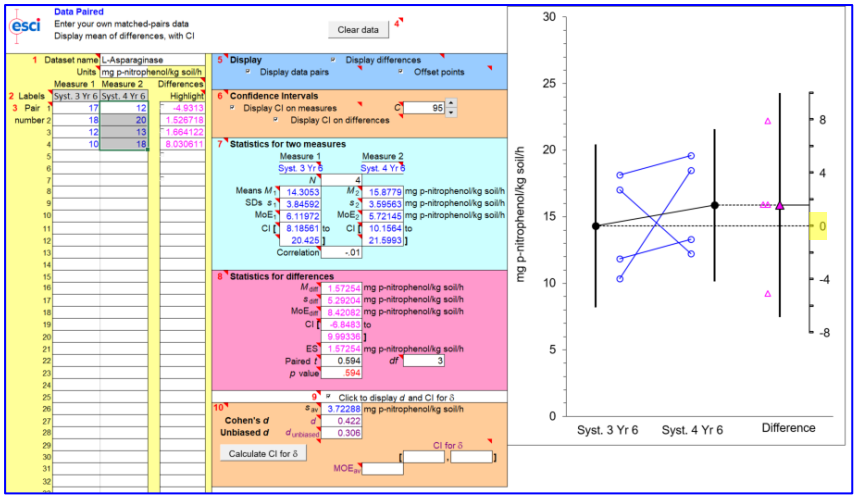
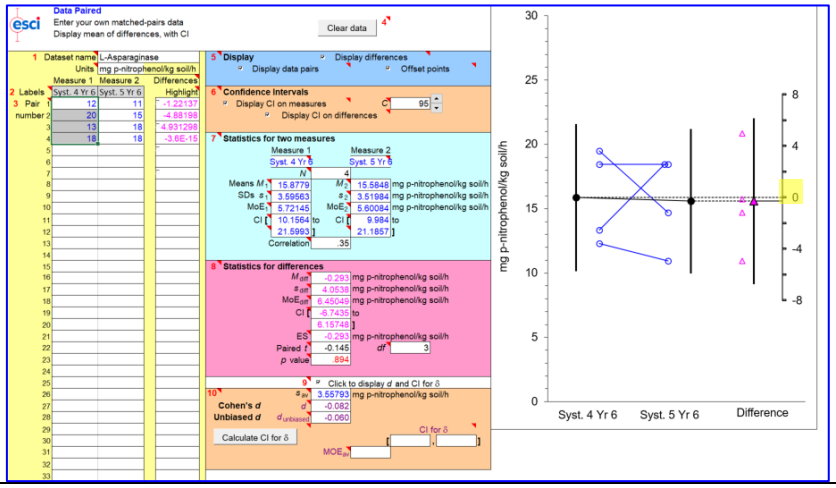
Supplemental Figure 9 (A-C)

Comparison of L-ASPARAGINASE activity between the three Cover Crop types After 6 years

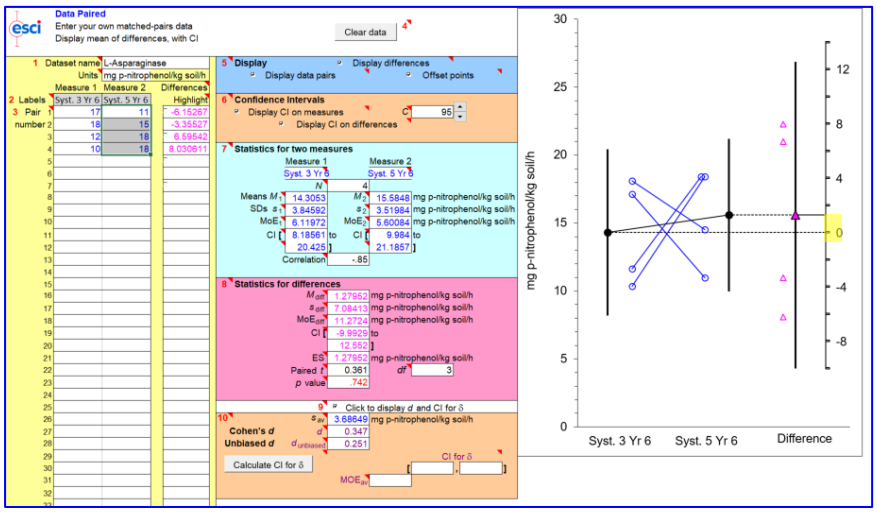
9A. System 3 (Compost + Legume-rye annually) versus System 4 (Compost + Mustard annually)



9B. System 4 (Compost + Mustard annually) versus System 5 (Compost + Rye annually)



9C. System 3 (Compost + Legume-rye annually) versus System 5 (Compost + Rye annually)



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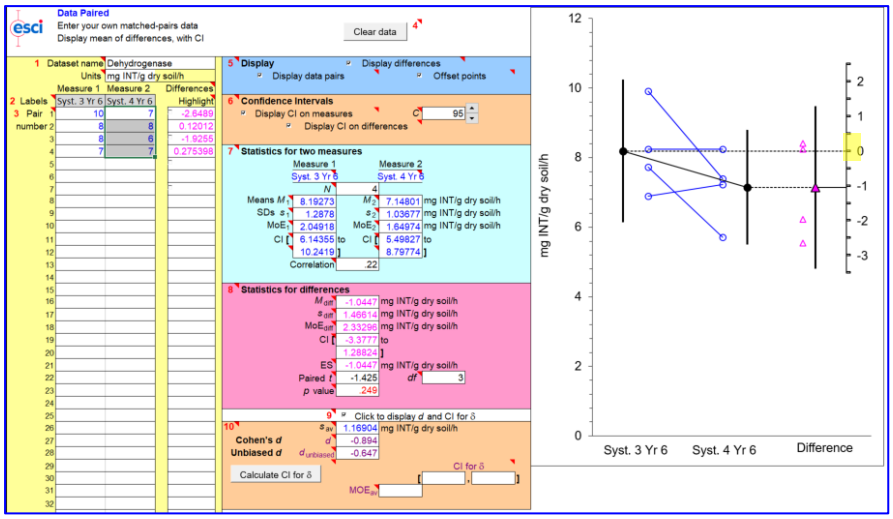
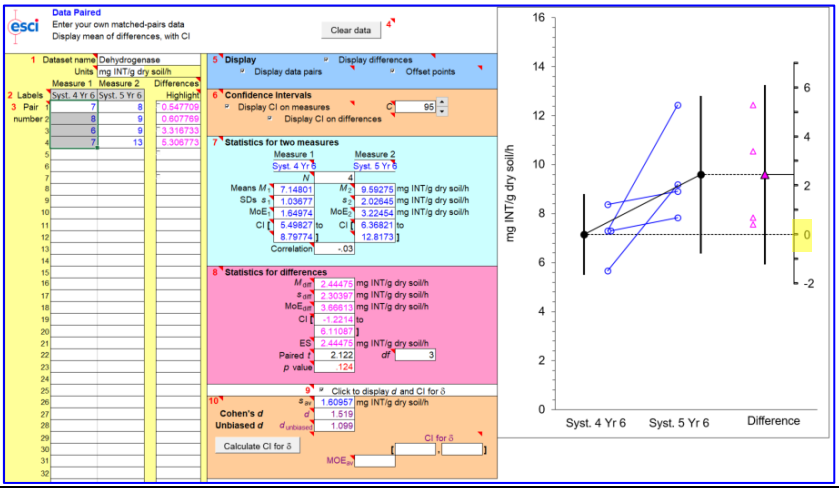
Supplemental Figure 10 (A-C)

Comparison of DEHYDROGENASE activity between the three Cover Crop types After 6 years

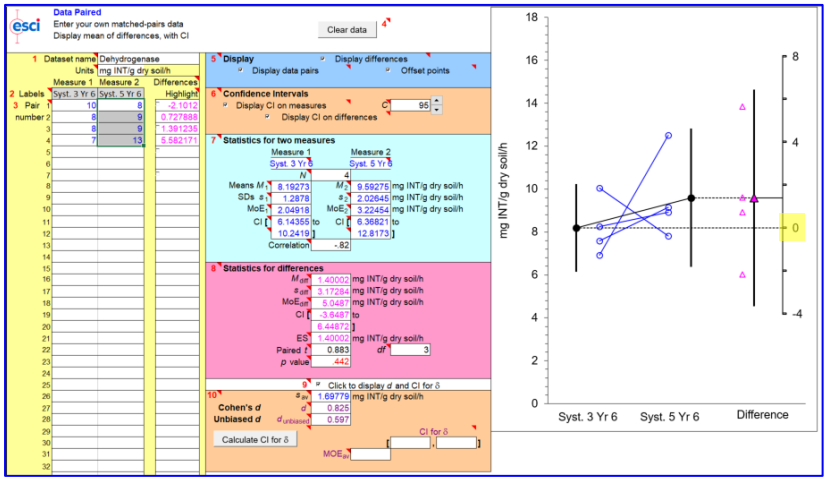
10A. System 3 (Compost + Legume-rye annually) versus System 4 (Compost + Mustard annually)



10B. System 4 (Compost + Mustard annually) versus System 5 (Compost + Rye annually)



10C. System 3 (Compost + Legume-rye annually) versus System 5 (Compost + Rye annually)



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