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Authors

Schmidt, Jennifer E
Vannette, Rachel L
Igwe, Alexandria
et al.

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Effects of agricultural management on rhizosphere microbial structure and function in processing

tomato

Running title: Management effects on tomato rhizosphere

Jennifer E. Schmidt[a], Rachel L. Vannette[b], Alexandria Igwe[b], Rob Blundell[c], Clare L. Casteel[c], Amélie

C.M. Gaudin[a#]

[a]Department of Plant Sciences, University of California at Davis, One Shields Avenue, Davis, CA 95616

[b]Department of Entomology and Nematology, University of California at Davis, One Shields Avenue, Davis, CA 95616

[c]Department of Plant Pathology, University of California at Davis, One Shields Avenue, Davis, CA 95616

#Corresponding author:

    Email: agaudin@ucdavis.edu

    Phone: 530-752-1212

    Mailing address: Department of Plant Sciences
        University of California Davis
        1 Shields Avenue
        Plant and Environmental Science Building
        Davis CA 95616

Abstract

2 Agricultural management practices affect bulk soil microbial communities and the functions they carry

3 out, but it remains unclear how these effects extend to the rhizosphere in different agroecosystem

4 contexts. Given close linkages between rhizosphere processes and plant nutrition and productivity,

5 understanding how management practices impact this critical zone is of great importance to optimize

6 plant-soil interactions for agricultural sustainability. A comparison of six paired conventional-organic

7 processing tomato farms was conducted to investigate relationships between management, soil

8 physicochemical parameters, and rhizosphere microbial community composition and functions.

9 Organically managed fields were higher in soil total N and NO_3-N, total and labile C, plant Ca, S, and Cu,

10 and other essential nutrients, while soil pH was higher in conventionally managed fields. Differential

11 abundance, indicator species, and random forest analyses of rhizosphere communities revealed

12 compositional differences between organic and conventional systems and identified management-specific

13 microbial taxa. Phylogeny-based trait prediction showed that these differences translated into more

14 abundant pathogenesis-related gene functions in conventional systems. Structural equation modeling

15 revealed a greater effect of soil biological communities than physicochemical parameters on plant

16 outcomes. These results highlight the importance of rhizosphere-specific studies, as plant selection likely

17 interacts with management in regulating microbial communities and functions that impact agricultural

18 productivity.

19 **Importance**

20 Agriculture relies in part on close linkages between plants and the microorganisms that live in association

21 with plant roots. These rhizosphere bacteria and fungi are distinct from microbial communities found in

22 the rest of the soil and are even more important to plant nutrient uptake and health. Evidence from field

23 studies shows that agricultural management practices such as fertilization and tillage shape microbial

24 communities in bulk soil, but little is known about how these practices affect the rhizosphere. We

25 investigated how agricultural management affects plant-soil-microbe interactions by comparing soil

26 physical and chemical properties, plant nutrients, and rhizosphere microbial communities from paired

27 fields under organic and conventional management. Our results show that human management effects

28 extend even to microorganisms living in close association with plant roots and highlight the importance of

29 these bacteria and fungi to crop nutrition and productivity.

30

31 **Introduction**

32 Soil microbial communities mediate the provision of many ecosystem services by soils and are

33 increasingly recognized as fundamental regulators of plant and environmental outcomes of

34 agroecosystems. Agricultural practices such as nutrient inputs and tillage have been shown to shape bulk

35 soil microbial communities and functions across spatial and temporal scales (1–4). Comparisons of bulk

36 soil under different management strategies, i.e. organic (nutrients provided from sources other than

37 synthetic inputs) vs. conventional management have revealed effects on soil properties that in turn drive

38 variation in microbial communities at small and intermediate scales (5–8). Small-scale studies designed to

39 minimize environmental heterogeneity, such as long-term experiments on a single site, show strong

40 effects of management on soil physicochemical parameters (9, 10), microbial biomass (9), and habitat-

41 specific bacterial and fungal taxa (11). At an intermediate spatial scale, such as paired fields within a

42 region, contextual variables such as climate, soil type, and cropping system largely influence the soil

43 physicochemical parameters and microbial processes that differ between conventional and organic fields.

44 Organically managed processing tomato fields in California have higher levels of organic carbon,

45 microbial abundance and diversity, and N mineralization potential compared to conventional, while soils

46 under conventional management have higher inorganic N pools and salinity (7). However, these studies

47 often have not extended to the rhizosphere, and the studies that have done so have not found universal

48 predictors of rhizosphere community assembly across contexts and scales (4, 12–14).

3

49   While bulk soil communities affect recruitment and assembly of rhizosphere microbial communities (15),

50   soil under the influence of plant roots represents a unique environment that must be studied separately

51   (16). The rhizosphere is a hotspot of interactions where dynamic relationships between plant roots and

52   soil microbial communities occur, allowing bacteria and fungi to break down and cycle organic matter

53   and release nutrients (17), promote plant growth via direct and indirect mechanisms (18), and suppress

54   pathogens (19). While linking agricultural management to large-scale outcomes such as nutrient fluxes or

55   ecosystem services requires analysis of bulk soil properties and processes, understanding the complex

56   relationship between management practices and plant nutrition and productivity necessitates shifting

57   focus to the rhizosphere (20). Some evidence suggests that management can affect the ecosystem-level

58   functions carried out by bulk soil microbial communities through impacts on microbial diversity (21), but

59   the unique chemistry and microbial communities found in the rhizosphere (22) are more closely linked to

60   plant outcomes of agricultural importance (23). Because rhizosphere soil is shaped by complex

61   interactions between plant and bulk soil processes, the effects of agricultural management on rhizosphere

62   communities and the functional implications are not always easy to predict.

63   The few studies that have addressed this question have concluded that differences in bulk soil microbial

64   and protist communities do carry over to some extent to rhizosphere communities (22, 24). However,

65   such studies have frequently been conducted on long-term research stations (22, 24), leaving open the

66   questions of scale and context. Do management effects on rhizosphere microbial communities extend to

67   an intermediate scale, such as paired fields within a region? If so, what soil properties are most closely

68   linked to microbial variation, and how do differences in rhizosphere microbial communities influence

69   plant health and productivity?

70   A regional-scale study of paired organic and conventional processing tomato fields in Northern California

71   was conducted to i) characterize impacts of agricultural management on rhizosphere microbial

72   community composition in California processing tomato agroecosystems at an intermediate spatial scale,

73   ii) identify how taxonomic shifts affected predicted metabolic and ecological functions carried out by

4

74   these communities, and iii) explore the effects of management-induced microbial variation on crop

75   nutrition and productivity. To address the first objective of identifying variation in rhizosphere microbial

76   communities, we employed three complementary approaches: differential abundance, indicator species,

77   and random forest analyses. Differential abundance analysis of microbial communities adapts RNA-seq

78   methodology used for gene expression to identify taxa whose abundance varies significantly among

79   groups of samples (25). Indicator species analysis, an alternative approach, detects taxa preferentially

80   associated with a given habitat or sample group based on a combination of specificity and fidelity rather

81   than relative abundance alone (26). Random forest analysis (27), a machine learning method, approaches

82   the microbe-sample group linkage from the opposite direction than the differential abundance and

83   indicator species approaches, identifying key taxa whose abundance can be used to assign samples to the

84   appropriate group.

85   The second objective, determining whether agricultural management induces shifts in rhizosphere

86   microbial functions, was addressed using phylogeny-based trait prediction. This method predicts

87   metagenomic data such as genes involved in key agroecological functions from 16S amplicon sequencing

88   data (28). Structural equation modeling (SEM), a statistical technique to test hypothesized relationships

89   among variables (29), was used to address our final objective of exploring linkages between soil

90   properties, microbial communities, and plant nutrition and productivity. We hypothesized that rhizosphere

91   community structure and function would differ between conventional and organic systems and that

92   divergent microbial communities would relate to variation in plant traits within and between fields.

93   **Results**

94   *Site and management drive variation in soil and plant variables*

95   Site had a stronger influence on bulk soil and plant variables than management category (organic vs.

96   conventional) (site R^2=0.54, p=0.001; management R^2=0.17, p=0.001) and the site x management

97   interaction was significant (R^2=0.09, p=0.001). Two principal components (PCs) explained 41.99% (PC1)

5

98   and 21.93% (PC2) of variation among samples, respectively (Figure 1). Samples tended to cluster

99   primarily by site along PC1, which was affected by numerous plant and soil nutrients, and secondarily by

100 management within each site. PC2 was primarily influenced by plant Cu, Mg, and Mn as well as soil Mg

101 and NO_3-N (Figure 1, Table 1).

102 Management system significantly affected soil physicochemical variables ($p<0.001$). Soil parameters that

103 were higher in organically managed fields included total N ($p<0.001$), C ($p<0.001$), NO_3-N ($p=0.008$),

104 Olsen-extractable P ($p=0.008$), K ($p=0.0087$), Na ($p<0.001$), organic matter (OM) ($p=0.0022$), and

105 permanganate-oxidizable carbon (PoxC) ($p<0.001$), while pH was higher in conventionally managed

106 fields ($p=0.044$). Soil physicochemical properties were highly correlated with one another (Table 2).

107 Magnesium was correlated with only NO_3-N, cation exchange capacity (CEC) and pH, but other

108 macronutrients and key soil properties tended to vary together. Management also affected plant nutrients

109 ($p<0.001$), many of which were correlated with one another (Table 3). Concentrations of Ca ($p=0.0004$),

110 S ($p<0.001$), and Cu ($p<0.001$) were all higher in plants from organically managed fields.

111 *Rhizosphere microbial community composition responds to management practices*

112 The species composition of both bacterial and fungal rhizosphere communities varied according to site

113 and management (Figure 2), and these effects were also observed when phylogenetic relatedness of

114 bacterial communities was considered (Figure S1 in the supplementary material). Tests of multivariate

115 homogeneity of group dispersions (betadisper function of the vegan package) showed that dispersions did

116 not differ among sites or management types (both $p>0.05$). Management influenced rhizosphere microbial

117 communities, but to different extents depending on the site identity (Figure 2, site x management

118 interaction bacteria $R^2=0.12$, $p<0.01$; fungi $R^2=0.10$, $p<0.01$). Management accounted for the greatest

119 proportion of variation (53%) in bacterial communities at the MR site ($R^2 = 0.53$, $p=0.02$), slightly more

120 than at the PF site ($R^2=0.43$, $p=0.02$) and nearly three times as much as at the RR site ($R^2=0.19$, $p=0.01$).

121 Fungal communities were also affected by management, which accounted for 22% of variation at the MR

122 site ($R^2=0.22$, $p=0.02$), 38% at the RR site ($R^2=0.38$, $p=0.01$), and 43% at the PF site ($R^2=0.43$, $p=0.02$).

6

123   Bacterial diversity was affected by the site x management interaction (p<0.001). The Shannon index was

124   higher in organically managed fields than conventionally managed fields at all sites except the MR site

125   (Table 4). Fungal diversity was affected by site (p<0.001) and management (p<0.001), but not the

126   interaction. Fungal diversity was higher in organically managed fields at all sites, and higher at the PF site

127   than RR or MR (Table 4).

128   Forty-eight bacterial amplicon sequence variants (ASVs) differed in abundance between the rhizospheres

129   of conventionally and organically managed plants at the α=0.01 level (Figure 3a). ASVs more abundant

130   in organically managed rhizospheres included two members of the genus *Pseudomonas*, while ASVs

131   more abundant in conventionally managed rhizospheres included six members of the genus

132   *Flavobacterium* and three members of the genera *Devosia* and *Lysobacter*. An ASV belonging to the

133   genus *Pseudomonas* had the highest relative abundance in organically managed fields, and an ASV

134   belonging to the genus *Chryseobacterium* had the highest relative abundance in conventionally managed

135   fields.

136   Nineteen fungal ASVs differed in abundance between management systems at the p=0.01 level, only one

137   of which was more abundant in conventionally managed fields (Figure 3b). ASVs more abundant in

138   organically managed plant rhizospheres included three members of the genus *Holtermanniella*, three

139   members of the genus *Mucor*, and two members of the genus *Pyrenochaetopsis*. The ASV more abundant

140   in conventionally managed rhizospheres was identified as *Plectosphaerella cucumerina*. *Mucor hiemalis*

141   was most abundant in organic systems relative to conventional.

142   Since system management has a strong impact on multiple soil properties, we conducted redundancy

143   analysis (RDA) with forward selection to identify which soil physicochemical properties have the greatest

144   influence on rhizosphere bacterial and fungal community composition. After site and management, Ca

145   was the most significant driver of both bacterial and fungal community composition. Bacterial community

146   composition also responded to Mg levels, while fungi were significantly influenced by Na and K.

7

147   *Indicator species of rhizosphere communities differ between systems*

148   Indicator species analysis showed 57 system-specific bacterial ASVs: 35 with the conventional system

149   and 22 with the organic system (Table S3). Members of the genera *Flavobacterium* (8), *Pedobacter* (4),

150   *Lysobacter* (3), and *Pseudomonas* (3) had by the greatest number of sequences in the conventional system

151   and *Pseudomonas* (4) in the organic system. Fewer fungal indicator taxa were discovered, with only four

152   fungal ASVs associated with the conventional system but 17 with the organic system. The four ASVs

153   associated with the conventional system came from different genera, while *Holtermanniella* (6) and

154   *Mucor* (4) were the most represented indicator genera in the organic system. Fifteen of the 78 taxa

155   identified by indicator species analysis were also differentially abundant. Because the IndVal index

156   represents the probability of finding a given species in the environment of interest, taxa with a high

157   relative abundance in the environment will generally score high on the fidelity component of the IndVal

158   index. This was the case for *Flavobacterium* in the conventional system and *Pseudomonas*,

159   *Holtermanniella*, and *Mucor* in the organic system.

160   Random forest (RF) analysis was used to identify ASVs that could be used to discriminate between

161   management systems. ASVs belonging to the genera *Lysobacter* and *Gibellulopsis* had the greatest

162   impact on the mean decrease in accuracy and mean decrease in Gini coefficient of the random forest

163   model (Figure S2 in the supplementary material). Substantial overlap was observed between the results of

164   RF analysis and differential abundance analysis. Eleven of the twenty most significant ASVs from the RF

165   analysis had also been identified through differential abundance analysis, although ASVs such as

166   *Gibellulopsis* that had a significant impact on the RF model only slightly differed in abundance between

167   systems (Figure 3).

168   *Management induces changes in predicted rhizosphere bacterial functions*

169   Of the total number of genes predicted, 4.8% (169) differed in abundance between the rhizosphere of

170   organic and conventional plants. Of those genes, 79 were more abundant in the organic system and 90

8

171   were enriched in the conventional system. Functions corresponding to cellular processes including

172   quorum sensing, biofilm formation, and chemotaxis showed the greatest difference between systems, with

173   only two peroxisome functions upregulated in the organic system and 31 upregulated in the conventional

174   system (Figure 4). Genes with the highest relative abundance in the organic system were distributed

175   across a variety of functions, including ABC transporters (12), two-component systems (8), biosynthesis

176   of siderophores (5), starch and sucrose metabolism (5), and type I polyketide structures (5). A component

177   of the *trcR*/*trcS* two-component regulatory system, *trcR* (K07672), was up-regulated by the greatest ratio

178   in organic systems. Genes with greater relative abundance in the conventional system tended to be

179   associated with biosynthesis of amino acids (19), two-component systems (18), quorum sensing (10),

180   ABC transporters (9), and biofilm formation (9) (Figure 4).

181   *Structural equation modeling identifies key linkages among plant, soil, and microbial variables*

182   Hypothetical links between bulk soil physicochemical parameters, plant nutrition, rhizosphere microbial

183   communities, and plant biomass were tested using structural equation modeling (SEM) across

184   management systems (Figure 5a). Bacterial and fungal communities were represented by two vectors each

185   (PC1B, PC2B, PC1F, PC2F) that were derived from principal components analysis shown in Figure 2.

186   Plant biomass was most strongly positively correlated with plant P, which in turn was most strongly

187   correlated with fungi from the PC2F vector (Figure 5b). The taxa that contributed most to PC2F were

188   *Vishniacozyma victoriae* and an unidentified *Solicoccozyma* sp. Neither of these species were identified in

189   the differential abundance analysis (Figure 3b). Fungi from the PC1F vector had a slight positive

190   influence on plant Na and included ASVs classified as *Alternaria* sp*., Cryptococcus aerius*, and

191   *Plectosphaerella cucumerina*. PC1B, the first principal component of bacterial communities, was

192   negatively correlated with shoot C:N ratio; the three ASVs with the greatest contribution to this

193   component were a strain of *Pseudomonas* and two strains of *Stenotrophomonas*. The second principal

194   component of bacterial communities (PC2B) was slightly positively correlated with plant biomass, P, Na,

9

195   and C:N ratio; four of the five ASVs with the greatest contribution to this component were classified as

196   *Pseudomonas* sp.

197   The final SEM had a χ^2 test statistic of 1.907 with 3 degrees of freedom, giving a χ^2/v ratio of 0.64, root

198   mean square error of approximation (RMSEA) of 0.000 (90% confidence interval $0.000 \leq x \leq 0.195$),

199   comparative fit index (CFI) of 1.000, Tucker Lewis index (TLI) of 1.062, and standardized root mean

200   square residual (SRMR) of 0.016. A low χ^2/v ratio indicates a good model, although this test statistic does

201   not perform well with small sample sizes (30). The CFI and TLI model indices perform well with small

202   sample sizes and are above acceptable threshold (0.95 for a good model (31)). An SRMR less than 0.08

203   generally indicates that a model fits the data well (32).

204

205   **Discussion**

206   Our objectives were to explore how management practices implemented in organic and conventional

207   tomato production systems shape rhizosphere microbial composition, infer how taxonomic shifts affected

208   microbe-mediated functions, and identify linkages between management-induced shifts in soil

209   physicochemical parameters, rhizosphere microbial communities, and plant nutrition and productivity. In

210   support of our hypotheses, we identified specific taxa that differed in abundance between management

211   systems and predicted the functional implications of those shifts in community composition (Figure 3,

212   Figure 4, Figure S2). Some differentially abundant taxa were confirmed as indicator species that could be

213   used to distinguish communities between management systems. More importantly, phylogeny-based trait

214   prediction showed that management-induced differences in rhizosphere bacterial community composition

215   translated into agriculturally relevant outcomes, particularly with regard to plant nutrition and pathogen-

216   related functions such as quorum sensing and biofilm formation(33, 34) (Figure 4, Figure 5). Although

217   our techniques could not examine the contribution of fungi to predicted function, it is likely that observed

218   compositional shifts in fungal communities increase divergence in functional outcomes between systems.

10

219   Bacterial diversity was higher in the rhizospheres of organically managed plants at all sites except MR,

220   and fungal diversity was higher in the organic system across sites, consistent with other studies finding

221   increased microbial diversity under organic management (2, 7, 35–37). Numerous bacterial ASVs

222   belonging to the genus *Pseudomonas*, which contains members known to possess plant-growth-promoting

223   properties (18, 19), had a higher relative abundance in organic systems (Figure 3a). Sixteen of the 17

224   differentially abundant fungal ASVs were found at higher abundance in the rhizosphere of plants growing

225   in the organic system; these included numerous members of the genera *Holtermanniella* and *Mucor*

226   (Figure 3b). *Holtermanniella* is a small, cold-tolerant genus of potentially parasitic fungi (38) that

227   includes species able to metabolize diverse carbon compounds and generate unique fatty acid profiles

228   (39). *Mucor* are a genus of starch decomposing fungi (40) that are capable of metabolizing a wide range

229   of complex carbohydrates (41). Although a long-term comparison of conventional and organic

230   management found no difference in the relative abundance of *Mucor* sp. in bulk soils (42)**,** potential shifts

231   in the rhizosphere have not been shown. In addition, predicted potential community functions also

232   differed between soils under different management systems. Although our approach relies on predicted

233   potential (DNA-based) functions rather than genomic or transcriptomic information from the strains

234   found at these sites, tax4fun performs well in comparison with shotgun metagenomic data from soils (28),

235   suggesting that broad patterns may be informative. Bacterial community shifts in the rhizospheres of

236   organically managed plants were associated with a higher abundance of predicted genes involved in

237   starch and sucrose metabolism and biosynthesis of siderophores, which can increase the availability of

238   micronutrients such as iron (Figure 4). Other enzymes with high relative abundance in the organic system

239   catalyze reactions involved in the metabolism of tyrosine, carotenoids, and other complex organic

240   compounds (Figure 4).

241   Rhizosphere diversity was generally lower under conventional management, and community composition

242   and functions were notably different. ASVs belonging to the genera *Flavobacterium*, *Devosia*, and

243   *Lysobacterium* had higher relative abundances in the conventional system. The *Flavobacterium* genus has

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244   been found elsewhere to increase in abundance in response to six years of intensive organic vegetable

245   production (43), suggesting that individual species within the genus may respond differently to

246   conventional and organic management. Members of *Lysobacterium* have been shown to degrade complex

247   aromatic compounds (44). *Plectosphaerella cucumerina*, a known pathogen that causes rots on a variety

248   of horticultural species (45), was the only fungal ASV found to be more abundant in the conventional

249   system. Perhaps due to the greater abundance of this pathogen, functions upregulated in the conventional

250   system included genes related to quorum sensing and biofilm formation (Figure 4).

251   Management practices and sites had strong influence on soil chemical properties, which in turn affected

252   bacterial and fungal community composition. Forward selection revealed that the two kingdoms

253   responded to different sets of soil physicochemical parameters: bacterial community composition was

254   affected by Ca and Mg, while fungal community composition was affected by Ca, Na, and K. These

255   predictors are notably different from variables commonly accepted as important for microbial community

256   composition, such as organic matter (46, 47), pH (48, 49), and N. The failure of organic matter and N to

257   predict microbial community structure is surprising at first glance, given that scarce C and N availability

258   can limit rates of microbial growth and functions such as mineralization, and that the abundance of N-

259   cycling microbial taxa often varies with C and inorganic N species. However, this result is consistent with

260   multiple studies showing no effect of N on microbial community composition (50–52). Agricultural

261   management might outweigh the effects of variation in these parameters, since Ca and Mg were not

262   affected by management. It may also be that low variation in organic matter, pH, and soil N within the

263   context of this study reduced the ability of these parameters to explain variation in community

264   composition (Table S2 in the supplementary material).

265   Soil Ca and Na have similarly appeared elsewhere as significant predictors of microbial community

266   composition. In another comparison of management systems, soil Ca was higher in soils receiving

267   organic amendments than synthetic amendments and was among the parameters correlated with microbial

268   community composition (53). Ca was also a primary driver of microbial community composition in a

12

269   multi-year study of a soil amended with composted tannery sludge (54). Salinity frequently drives

270   variation in microbial community composition, especially in irrigated systems, although most commonly

271   when a stronger salinity gradient is present due to environmental filtering based on salinity tolerance (55–

272   57).

273   SEM tied together this observed variation in microbial community composition with soil and plant

274   variables and tested a hypothetical model linking plant and soil biological and physicochemical

275   parameters with plant biomass (Figure 5). Management was not retained in the final model, suggesting

276   that management effects were indirect and captured by other included variables at these sites. Other

277   studies have similarly found that soil type and physicochemical parameters affect microbial community

278   composition and catabolic functions more than long-term agricultural management practices (58). Within

279   this study, it appears that rhizosphere microbial communities were more closely linked to differences in

280   bulk soil properties created by management systems than to the management practices themselves.

281   SEM revealed a greater relative influence of rhizosphere biological communities than bulk soil

282   physicochemical characteristics on plant nutrient content and biomass (Figure 5). A strong indirect

283   linkage was observed between microbial communities and plant biomass: fungal community composition

284   was strongly positively correlated with plant P, which in turn strongly correlated with shoot biomass

285   (Figure 5). The link between plant P and fungal communities is particularly striking given the absence of

286   sequences belonging to the phylum Glomeromycota, which contains mycorrhizal fungi (data not shown).

287   The lack of mycorrhizal sequences may be partly explained by choice of amplicon or primer bias (59).

288   Since the length of the amplified region differs for mycorrhizae compared to the more abundant

289   Ascomycota and Basidiomycota (60); it is unlikely that mycorrhizae were truly absent from all samples.

290   Nonetheless, even non-mycorrhizal fungi can improve plant P status through solubilization,

291   mineralization, and direct transfer of phosphate (61). Genera such as *Aspergillus* and *Penicillium* release

292   organic acids that can solubilize phosphate, potentially rendering it available for direct uptake by plants or

293   mycorrhizae (62).

13

294   PC2B was slightly positively correlated with plant Na and PC1B was negatively correlated with C:N

295   ratio. The correlation between bacterial community composition and plant Na could be the direct effect of

296   microbial interference in plant metabolism, or changes in soil parameters could foster unique microbial

297   communities and also increase plant Na. While limitations of the measured data do not allow us to

298   distinguish between these explanations in this context, microbial influence on plant Na has been reported

299   elsewhere; certain bacterial strains are capable of plant tissue-specific regulation of sodium transporters

300   that increases salt tolerance in Arabidopsis (63), while other bacterial strains reduce salt accumulation in

301   salinity-stressed plants (64). A negative correlation with C:N ratio indicates that the bacterial populations

302   improved plant N content, a result that could be due to increased N availability via N fixation or

303   mineralization of organic matter.

304   This study identified rhizosphere microbial taxa and functions affected by agricultural management and

305   illuminated unexpected linkages between soil, microbes, and crop nutrition and productivity, but

306   compelling questions remain. Organic certification encompasses a diverse set of management practices

307   and variation in cover crop species, green manure inputs, or crop rotation complexity and duration likely

308   lead to diverse effects on soil microbes. To translate the broad, extensive conventional-organic literature

309   into tangible recommendations, future studies should focus on causal relationships between specific

310   inputs or techniques and key soil physicochemical parameters. This could be achieved in part by

311   employing SEM with a much larger dataset (a sample size of at least 200 (65) and data satisfying the

312   requirement of multivariate normality (66)) to allow the incorporation of additional variables (e.g. crop

313   genotype, N fertility source and rate, tillage) and improve the predictive power of the model. Such

314   analysis would add nuance to the results of this study and enable the development of management

315   systems that foster agricultural productivity by maximizing beneficial plant-soil-microbe interactions in

316   the rhizosphere.

317   Our results add an additional layer of complexity to previous investigations of the effects of agricultural

318   management on microbial communities. Others have noted the importance of scale in determining how

14

319   soil properties relate to microbial community composition or function, as geographic scale alters the

320   relative importance of factors such as environmental heterogeneity and distance that influence microbial

321   distribution (67, 68). We emphasize the importance of integrating plants and rhizosphere processes into

322   these discussions of microbial biogeography, particularly at intermediate scales, as plants exert strong

323   influence on rhizosphere communities and may modulate management effects on rhizosphere

324   communities. Management of plant-microbe-soil interactions in the rhizosphere is a critical step toward

325   building more resource-efficient and resilient agricultural systems, and our study indicates that soil

326   management has strong and consistent effects on landscape-level variation in the rhizosphere composition

327   and predicted function.

328   **Materials and Methods**

329   *Sample collection*

330   Samples were collected from 6 paired fields under conventional and organic management on Yolo Silt

331   Loam during the 2017 growing season (details of sites and management practices can be found in Table

332   S1 in the supplementary material). Plant and soil samples were collected ~ 6 weeks after transplanting on

333   the same date at paired fields. Samples were taken from six locations per field (two on the exterior

334   margins of the field and four internal). At each location, two entire plants were excavated and shoot and

335   root samples were separated by clipping at the base of the shoot. A bulk soil sample was collected from

336   the upper 10 cm of soil immediately adjacent to each plant. Roots were separated from bulk soil, stored in

337   paper bags and transported to the lab on ice. Twelve root fragments from each plot (6 from each

338   individual plant) were pooled and rhizosphere soil was collected using a shaking wash in an 0.9%

339   NaCl/0.01% Tween 80 (v/v) solution followed by centrifugation. Because this volume of soil was

340   insufficient for full textural and nutrient analysis, we assumed that rhizosphere soil characteristics such as

341   texture, organic matter, etc. would be similar to the parameters measured for the corresponding bulk soil.

342   Shovels and other sampling implements were cleaned thoroughly between samples. The remaining roots

343   and shoots were dried at 60°C and weighed.

15

344   *Plant and soil analysis*

345   Dried bulk soil samples and aboveground dried biomass were homogenized and analyzed for total

346   nitrogen (N) and carbon (C) via combustion analysis (69). Soil nitrate was measured using a flow

347   injection analyzer (70), soil extractable phosphorus (P) was determined according to Olsen and Sommers

348   (71), and other soil nutrients were measured using ICP-AES (72). Soil organic matter content was

349   determined via the loss-on-ignition method (73). Soil pH was measured on a saturated paste extract. Bulk

350   soil properties can be found in Table S2 in the supplementary material.

351   Dried aboveground biomass was ground thoroughly to pass a 2 mm sieve. Plant leaf samples were

352   analyzed for N, P, K, Ca, Mg, Mn, Fe, Cu, B, and Zn at the Agricultural Analytical Services Lab of

353   Pennsylvania State University. Total N was analyzed via combustion (74), and concentrations of the

354   remaining elements were determined via hot block acid digestion (75).

355   *Microbial community analysis*

356   DNA was extracted from rhizosphere samples using the MoBio PowerSoil Kit (Qiagen). At least 5 ng of

357   DNA from each sample was sent for library prep and sequencing using MiSeq at Dalhousie IMR

358   facility**.** The V4-V5 region of the 16S rRNA region was sequenced to characterize bacterial communities

359   and the ITS region of the rRNA gene was sequenced to characterize fungal communities (76,

360   77). Negative controls were also extracted and submitted, but no reads were recovered. All statistical

361   analyses were carried out using R software (78). Reads were error-corrected and assembled into amplicon

362   sequence variants (ASVs) using DADA2 v.1.8 (79), with taxonomy assigned using SILVA v.128 for

363   bacteria (80) and UNITE database (2017 release) for fungi (81). Taxa without a taxonomic assignment, or

364   assigned to Archaea, mitochondria, or chloroplasts were removed from this dataset. Those not assigned to

365   the kingdom Fungi were removed from the fungal dataset. Sequence abundance was rarefied to 15,310

366   sequences per sample for bacteria and 13,000 per sample for fungi and all samples approached saturation.

16

367   Sequencing data is available in the NCBI SRA data repository under the project accession number

368   PRJNA539989.

369   Non-metric multidimensional scaling (NMDS) was used to ordinate samples in two-dimensional space

370   (*ordinate* function of the phyloseq package using *method = "NMDS"*). Two outliers were removed from

371   this and subsequent analyses in order to minimize the stress function. A second NMDS ordination was

372   performed based on weighted UniFrac distances (*distance* function of phyloseq package with *"wunifrac"*

373   command) to determine whether phylogenetic distance among samples was affected by site and

374   management. Shannon diversity was calculated for each sample using the *estimate_richness* function

375   (*measures = "Shannon"*) of the phyloseq package.

376   *Differential abundance of microbial taxa*

377   Differential abundance of bacterial and fungal in the rhizosphere of plants grown in organically and

378   conventionally managed systems was carried out using the DESeq2 package (25). Although applying this

379   analysis to compositional datasets obtained from sequencing microbial communities has been critiqued

380   (82), the method has been shown to be effective when library sizes are similar across groups and sample

381   size is small (<50 samples per group) (83), as was the case here. Sequences occurring in fewer than three

382   samples were filtered out prior to the analysis to avoid bias due to rare taxa (*filter_taxa* function of

383   phyloseq package). Dispersions were fit to the mean intensity using a gamma-family GLM by setting the

384   parameter *fitType= "parametric"* and significance was assessed using the Wald test with a significance

385   threshold of $\alpha=0.01$.

386   *Indicator species analysis*

387   Indicator species analysis was conducted to identify specific rhizosphere microbial taxa that were

388   associated with the conventional or organic system using the indicspecies package (84). Briefly, the

389   Indicator Value (IndVal) index was calculated for each ASV-system combination as the product of

390   specificity and fidelity indices (84). The highest IndVal index for each ASV was tested for significance

17

391   with 999 permutations (*multipatt* function of indicspecies package using *duleg = TRUE* and *control=*

392   *how(nperm=999)*. A Bonferroni correction was used to control the family-wise error rate at α = 0.01.

393   *Random forest analysis*

394   We complemented the indicator species analysis with a random forest approach, which identifies bacterial

395   and fungal ASVs that could be used to classify samples by management system through a machine

396   learning algorithm. Random forest analysis was conducted using the randomForest package (27). The

397   dataset was split into subsets for training (70% of observations) and validation (30% of observations).

398   Model parameters were adjusted to minimize the error rate, but the default parameters for ntree (*ntree =*

399   *500*) and mtry (mtry = √p, with p representing the number of model parameters) resulted in the lowest

400   error rate (6.52%). The classification accuracy was calculated to be 95%, indicating high prediction

401   accuracy. ASVs with the greatest contribution to the classification algorithm were identified according to

402   the highest scores for mean decrease in accuracy or mean decrease in the Gini coefficient (*importance*

403   function of randomForest package).

404   *Phylogeny-based functional trait prediction*

405   We determined potential shifts in rhizosphere microbial functions with management and soil properties

406   using functional trait prediction of 16S communities with the themetagenomics package (85). Briefly, this

407   package implements Tax4Fun (28) to predict functions from the KEGG Orthology database that are

408   associated with provided abundance tables, sample metadata, and phylogenetic information. Phylogeny is

409   assigned according to the SILVA rRNA database project (80). To identify functions that differed in

410   abundance between systems, predicted functions were subjected to differential abundance analysis using

411   the DESeq2 package. Parameters were identical to those described previously and the significance

412   threshold was set at α=0.01.

413   *Principal component analysis of plant and soil variables*

18

414   Principal component analysis (PCA) was used to reduce the dimensions of the multivariate dataset

415   containing scaled soil and plant variables, visualize samples in two-dimensional space, and calculate

416   factor loadings (*prcomp* function of stats package). Outliers for individual soil and plant variables were

417   identified with Grubb's test (*grubbs.test* function of outliers package) and removed from the dataset prior

418   to PCA. The multivariate homogeneity of group dispersions (*betadisper* function of vegan package) was

419   tested to determine whether variances differed among sampling sites. The effect of management on soil

420   and plant variables was tested with multivariate analysis of variance (MANOVA) using the *manova*

421   function of the stats package (78).

422   *Permutational multivariate analysis of variance*

423   Permutational multivariate analysis of variance was used to test the effect of the interaction between site

424   and management on microbial community composition (*adonis* function of vegan package), separately for

425   bacteria and fungi. If the interaction was significant, the magnitude of the management effect was then

426   tested within each site. If the interaction was not significant, PERMANOVA was used to test the relative

427   magnitude of site and management effects. Redundancy analysis (RDA) was conducted to identify soil

428   physicochemical properties with the greatest influence on rhizosphere microbial community composition.

429   Parameters that significantly explained variation in bacterial or fungal community composition were

430   identified using forward selection (*ordistep* function of vegan package).

431   *Structural equation modeling (SEM)*

432   SEM was used to test a hypothetical model linking soil, plant, and microbial variables that affect shoot

433   biomass (Figure 5a). Parameters included in the model were chosen using forward selection of a linear

434   model with shoot biomass as the response variable and all other soil, microbial, and plant parameters as

435   independent variables (*step* function of stats package) (78). The model was established using the *sem*

436   function of the lavaan package (86) and visualized with the semPlot package (87). The model was then

437   refined by sequentially removing variables with poor explanatory power ($R^2 < 0.50$). Management (organic

19

438   vs. conventional) was originally included as a variable but was ultimately removed because management

439   significantly and consistently decreased the fit statistics for the model, perhaps because the variables

440   retained in the model were good indicators of management differences.

441   Although the variables identified by forward selection (soil Na, soil Ca, plant P, plant C:N, plant Na)

442   were not consistent with a hypothesis of multivariate normality, sample size was too small to permit the

443   exclusion of outliers. The first two principal components of microbial species composition, which

444   accounted for 31% and 15% of bacterial variation (PC1B and PC2B respectively) and 26% and 21% of

445   fungal variation (PC1F, PC2F respectively), were used to represent microbial communities in the model

446   (Figure 5). The maximum likelihood (ML) method was used to estimate model fit test statistics. The

447   goodness of fit of the model was tested using standard model fit indices: the ratio of the chi-square

448   statistic to degrees of freedom (χ^2/v), Root Mean Square Error of Approximation (RMSEA), Comparative

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699   **Figure Legends**

700   **Figure 1: PCA of soil and plant variables measured in six processing tomato fields.** Soil

701   physicochemical parameters and plant variables separated primarily by site along PC1, which explained

702   42% of variation. Samples separated secondarily by management within site, and a significant site x

703   management interaction was observed.

704   **Figure 2: NMDS ordination of microbial communities sampled from the rhizosphere of processing**

705   **tomatoes.** Non-metric dimensional scaling based on Bray-Curtis dissimilarity matrices revealed that A)

706   bacterial and B) fungal communities separated primarily by site and secondarily by management.

707   **Figure 3: Differentially abundant microbial taxa.** A) 48 bacterial and B) 19 fungal taxa differed in

708   abundance between conventional and organic management systems at the $\alpha=0.01$ level. Colored bars

709   represent the natural logarithm of abundance of each taxa and gray bars represent the ratio of abundance

710   in the organic system to abundance in the conventional system. Multiple strains or species within genus

711   are shown. NA indicates that sequences could not be identified at the genus level.

712   **Figure 4: Differentially abundant functions.** Phylogeny-based trait prediction revealed 169 functional

713   genes that differed in abundance between the two systems at the $\alpha=0.01$ level, 79 of which were more

714   abundant in the organic system and 90 in the conventional system.

715   **Figure 5: Structural equation model linking soil, plant, and microbial variables.** A) A hypothetical

716   model linking soil, microbial, and plant parameters was tested using structural equation modeling. B) The

717   final SEM showed that microbial communities had a strong but indirect effect on plant biomass through a

718   positive correlation between fungal community composition and plant P. Soil Ca and Na affected fungal

719   communities more strongly than bacterial communities. Red represents soil variables, blue represents

720   microbial variables (principal components 1 and 2 extracted from PCA of bacterial and fungal

721   communities, respectively), and green represents plant variables. Dashed lines represent fixed parameters.

Table 1. Factor loadings of scaled soil and plant variables contributing to PC1 and PC2.

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	PC1	PC2
Soil N (total)	-0.243	-0.159
Soil C	-0.254	-0.159
Soil NO$_3$-N	-0.063	-0.202
Soil P (Olsen)	-0.186	-0.024
Soil K	-0.260	-0.132
Soil Na	-0.190	-0.161
Soil Ca	-0.215	-0.166
Soil Mg	-0.108	0.361
CEC	-0.227	0.219
SOM	-0.264	-0.050
pH	-0.186	0.120
PoxC	-0.221	-0.173
Shoot mass	-0.211	0.199
Root mass	0.025	0.025
Shoot:root ratio	-0.111	0.031
Plant C	-0.217	0.230
Plant N	-0.118	0.145
Plant C:N	-0.097	0.034
Plant P	-0.190	0.250
Plant K	-0.253	0.055
Plant Ca	-0.251	-0.074
Plant Mg	-0.112	0.347
Plant S	-0.213	-0.117
Plant Mn	0.165	0.284
Plant Cu	0.012	-0.356
Plant B	-0.185	-0.114
Plant Zn	0.248	-0.124
Plant Na	0.006	0.234

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Table 2. Correlations among soil physicochemical properties based on the Pearson correlation coefficient. * = p<0.05, ** = p< 0.01, *** = p<0.001.

	N	C	NO₃-N	P	K	Na	Ca	Mg	CEC	OM	pH	PoxC
N	1.00	0.96***	0.46***	0.66***	0.84***	0.79***	0.64***	-0.04	0.37**	0.82***	0.25	0.84***
C		1.00	0.26	0.50***	0.90***	0.71***	0.76***	-0.02	0.45***	0.90***	0.41**	0.89***
NO3-N			1.00	0.62***	0.32*	0.56***	0.22	-0.44***	-0.24	0.06	-0.29*	0.21
P				1.00	0.57***	0.55***	0.36**	0.13	0.35**	0.43**	0.12	0.35**
K					1.00	0.63***	0.88***	0.02	0.55***	0.85***	0.61***	0.79***
Na						1.00	0.53***	-0.13	0.23	0.55***	0.22	0.59***
Ca							1.00	-0.11	0.48***	0.76***	0.60***	0.67***
Mg								1.00	0.81***	0.25	0.51***	-0.06
CEC									1.00	0.68***	0.79***	0.36**
OM										1.00	0.55***	0.76***
pH											1.00	0.34**
PoxC												1.00

Table 3. Correlations among plant variables based on the Pearson correlation coefficient. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.
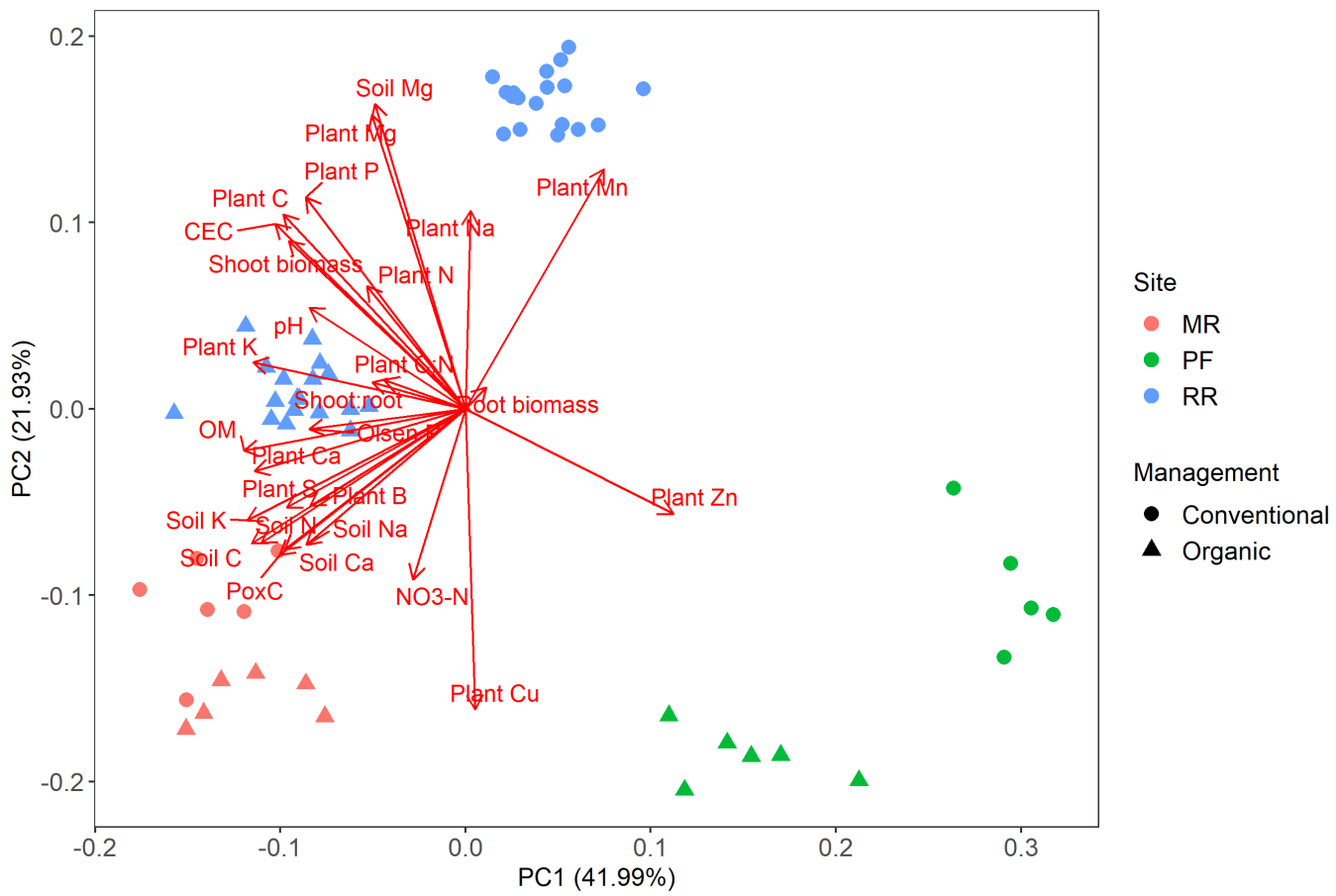
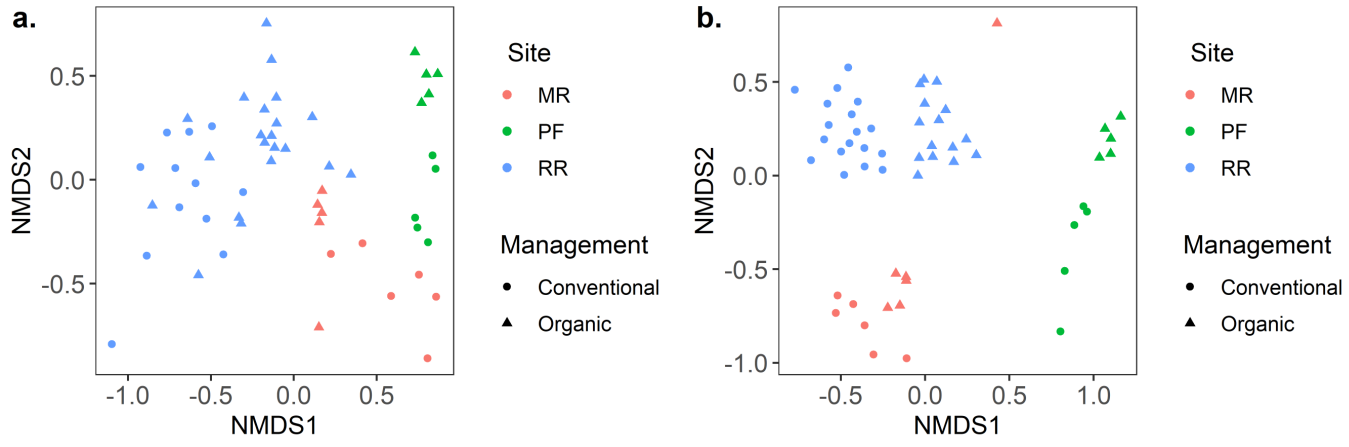|	Shoot biomass	C	N	P	K	Ca	Mg	S	Mn	Cu	B	Zn	Na	C:N	Root biomass	Shoot:root
Shoot biomass	1.00	0.75***	0.39**	0.86***	0.69***	0.55***	0.77***	0.48***	-0.03	-0.38**	0.27*	-0.74***	0.29*	0.28*	-0.01	0.33*
C		1.00	0.58***	0.80***	0.73***	0.54**	0.71***	0.32*	-0.04	-0.57***	0.35**	-0.87***	0.20	0.31*	-0.21	0.36**
N			1.00	0.53***	0.52**	0.33*	0.40**	0.06	0.03	-0.44***	0.13	-0.36**	0.40**	-0.59***	-0.17	0.30*
P				1.00	0.72***	0.53**	0.84***	0.42**	0.10	-0.47***	0.17	-0.69***	0.39**	0.17	-0.05	0.33*
K					1.00	0.78***	0.42**	0.55**	-0.43**	-0.13	0.60***	-0.74***	0.03	0.17	-0.09	0.38**
Ca						1.00	0.20	0.81***	-0.57***	0.27*	0.59***	-0.64***	-0.14	0.20	-0.21	0.42**
Mg							1.00	0.14	0.42**	-0.69***	-0.14	-0.52***	0.60***	0.19	0.00	0.24
S								1.00	-0.59***	0.45***	0.25	-0.47***	-0.08	0.31*	-0.19	0.26
Mn									1.00	-0.58***	-0.53***	0.20	0.37**	-0.16	0.07	-0.16
Cu										1.00	0.15	0.35**	-0.49***	0.00*	-0.10	0.02
B											1.00	-0.57***	-0.48***	0.26*	-0.01	0.20
Zn												1.00	0.08	-0.46***	0.18	-0.37**
Na													1.00	-0.31*	0.11	-0.02
C:N														1.00	0.00	-0.01
Root biomass															1.00	-0.62***

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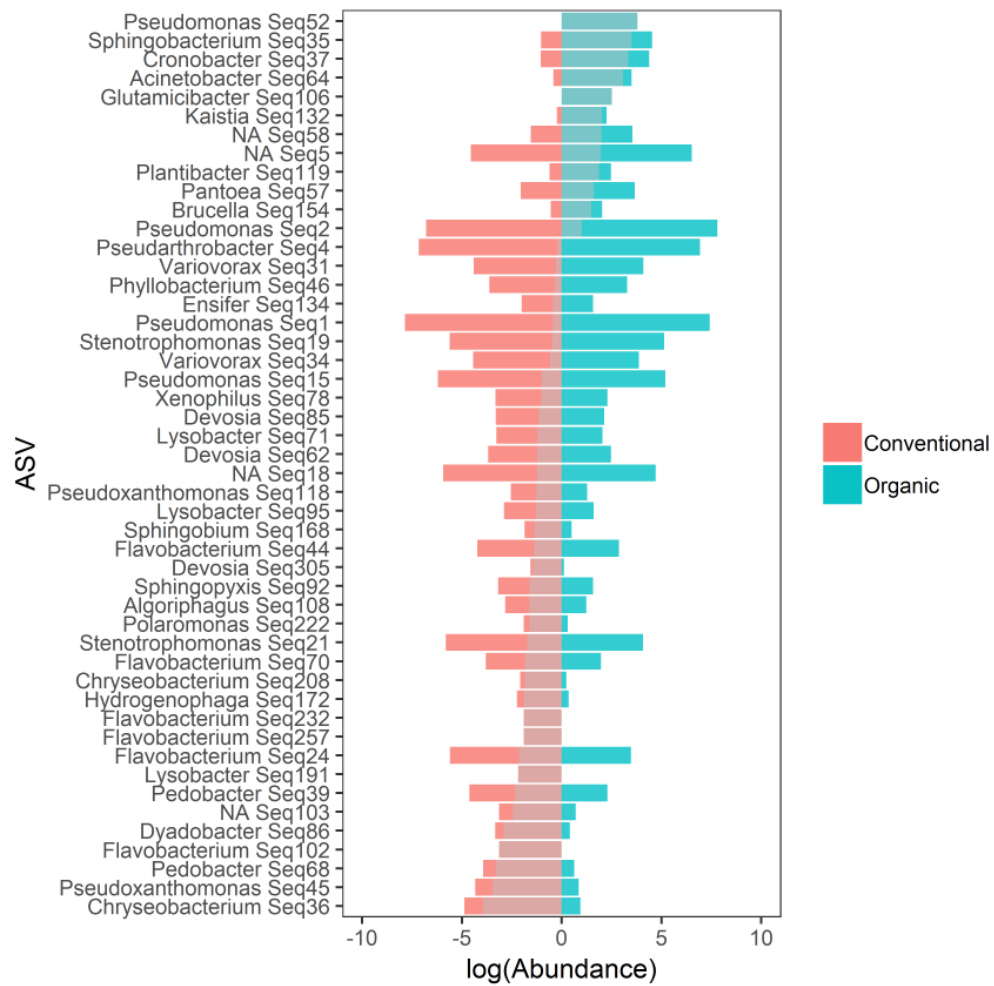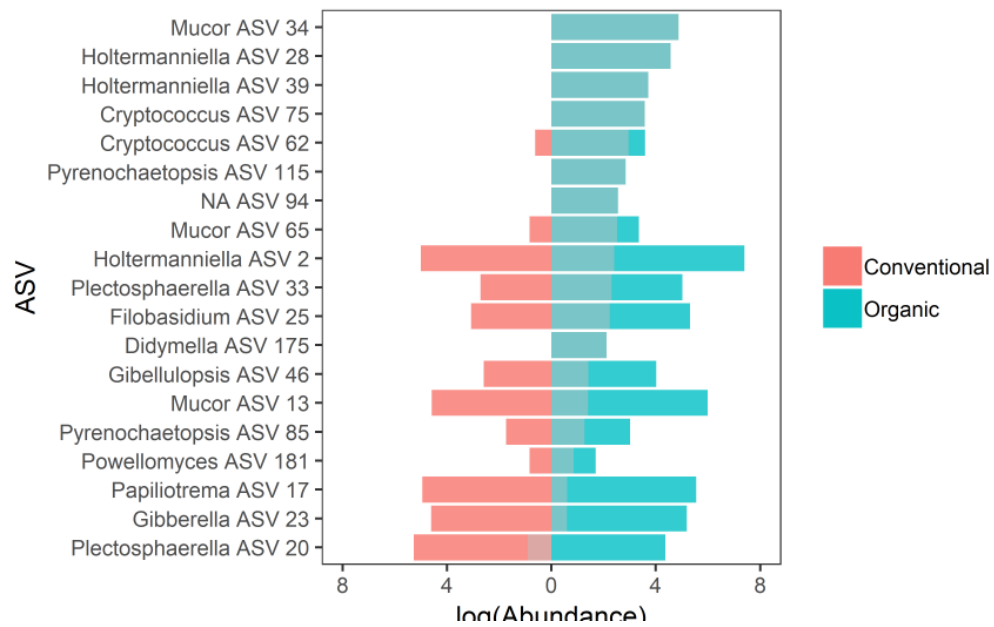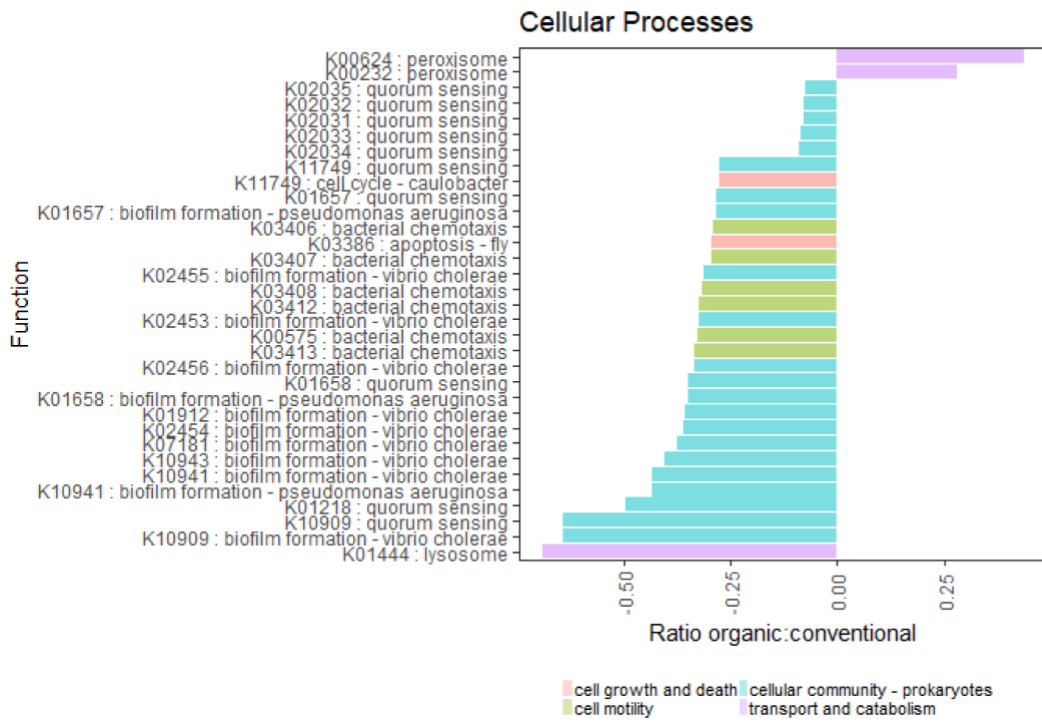|	
Shoot:root	1.00

Table 4. Alpha diversity of bacterial and fungal communities by site and management. Values reported are Shannon index ± standard error.

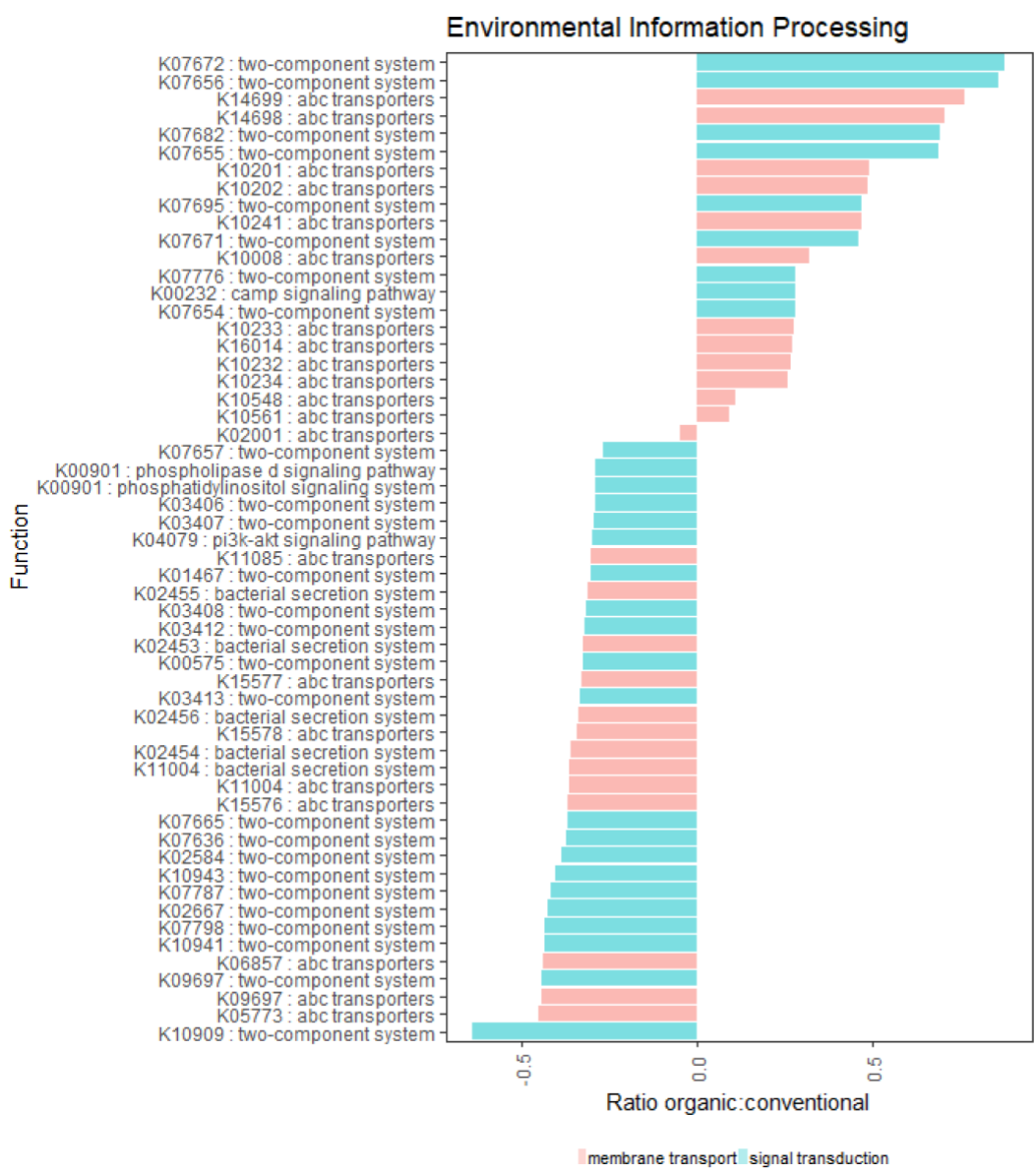|	MR		RR		PF	
	Conv.	Org.	Conv.	Org.	Conv.	Org.
16S	3.50 ± 0.14	2.90 ± 0.09	2.72 ± 0.05	2.99 ± 0.08	3.28 ± 0.08	3.58 ± 0.07
ITS	2.60 ± 0.08	2.62 ± 0.18	2.68 ± 0.08	3.12 ± 0.06	2.89 ± 0.16	3.32 ± 0.07

a.

b.

a.

b.

Cellular Processes

Environmental Information Processing

Genetic Information Processing

Organismal Systems

Bar chart titled "Organismal Systems". Y-axis labeled "Function" lists the following functions from top to bottom:
- K00232 : ppar signaling pathway
- K08598 : plant-pathogen interaction
- K08598 : nod-like receptor signaling pathway
- K04079 : signaling pathway
- K04079 : progesterone-mediated oocyte maturation
- K04079 : plant-pathogen interaction
- K04079 : nod-like receptor signaling pathway
- K04079 : estrogen signaling pathway
- K04079 : cell differentiation
- K04079 : antigen processing and presentation
- K00432 : thyroid hormone synthesis

X-axis labeled "Ratio organic:conventional" with values -0.2, 0.0, 0.2.

Legend: endocrine system, environmental adaptation, immune system

a.

b.