Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots

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Running title: Agricultural intensification affects root microbiota

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1   **Abstract** (200 words)

2   Root-associated microbes play a key role in plant performance and productivity, making

3   them important players in agroecosystems. So far, very few studies have assessed the impact

4   of different farming systems on the root microbiota and it is still unclear whether agricultural

5   intensification influences network complexity of microbial communities. We investigated the

6   impact of conventional, no-till and organic farming on wheat root fungal communities using

7   *PacBio SMRT sequencing* on samples collected from 60 farmlands in Switzerland. Organic

8   farming harboured a much more complex fungal network than conventional and no-till

9   farming systems. The abundance of keystone taxa was the highest under organic farming

10   where agricultural intensification was the lowest. The occurrence of keystone taxa was best

11   explained by soil phosphorus levels, bulk density, pH and mycorrhizal colonization. The

12   majority of keystone taxa are known to form arbuscular mycorrhizal associations with plants

13   and belong to the orders *Glomerales*, *Paraglomerales*, and *Diversisporales*. Supporting this,

14   the abundance of mycorrhizal fungi in roots and soils was also significantly higher under

15   organic farming. To our knowledge, this is the first study to report mycorrhizal keystone taxa

16   for agroecosystems, and we demonstrate that agricultural intensification reduces network

17   complexity and the abundance of keystone taxa in the root microbiota.

Introduction

Agricultural intensification is one of the most pervasive problems of the 21st century [1]. To keep pace with the ever-increasing human population, the total area of cultivated land worldwide has increased over 500% in the last five decades [2] with a 700% increase in the fertilizer use and a several-fold increase in pesticide use [3, 4]. Agricultural intensification has raised a wide range of environmental concerns, including poor nutrient-use efficiency, enhanced greenhouse gas emissions, groundwater eutrophication, degradation of soil quality, and soil erosion [4, 5]. Alternate farming systems such as conservation agriculture (e.g., no-till) and organic farming have been widely adopted to reduce such adverse environmental effects [6, 7]. Organic farmlands represent 2.5% of the total arable lands in Europe, and over 3.5% in Switzerland [8], although they may reduce yield and yield stability [9]. The adoption of no-till globally has increased by approximately 233% in the last decade and it is over 3% of the total arable lands in Switzerland [10]. These farming systems are adopted to maintain environmental sustainability and ecosystems services, and at the heart of ecosystem services lies the contribution of microbial communities [11–13].

Microbial communities play an indispensable role in ecosystems and render a wide range of services [12, 14–16]. In agroecosystems, microbes modulate a number of processes, including nutrient cycling, organic matter decomposition, soil aggregate stabilization, symbiotic and pathogenic interactions with plants, and thereby play an essential role in the productivity and sustainability of agroecosystems [5, 12, 17]. The agricultural intensity with high resource-use and low crop diversity can affect soil- and plant-associated microbiota, with subsequent impact on ecosystem services [18, 19]. Increasing adoption of no-till and organic farming also warrants an investigation of their effects on microbial communities. Previous studies comparing the effects of conventional, no-till and organic farming have

3

42   mostly focused on the soil microbiome [20–22], and our understanding of the impact of these

43   farming systems on root-associated microbiota is minimal.

44   Root-associated microbiota plays a key role in determining the above-ground

45   productivity [23, 24]. No-till farming may affect root architecture and root distribution in

46   soil, with a subsequent effect on microbial recruitment into the roots [25]. However, very few

47   studies have assessed the effect of no-tillage on root microbial communities, and the ones that

48   investigated root microbiota have only focused on root bacteria [26] or specific fungal

49   groups, including arbuscular mycorrhizal fungi (AMF) using traditional techniques [27, 28].

50   Furthermore, the impact of agricultural intensification on the overall root fungal communities

51   is still poorly understood [29, 30]. Plant root harbours a diverse assemblage of endophytic

52   fungi that form symbiotic, parasitic or pathogenic associations, and through such

53   associations, play a key role in plant diversity, community composition and performance

54   [31]. The widespread symbiosis of AMF and the array of benefits rendered by these fungi are

55   now well-established [32, 33]. Moreover, mycorrhiza like endophytes, *Piriformospra indica*,

56   also promote plant growth, stress tolerance and induce local and systemic resistance to

57   pathogens [34]. *Trichoderma* spp. have also been shown to enhance plant growth and

58   systemic resistance to plant pathogens [35]. Thus, the structure and composition of root

59   fungal communities play an important role in agroecosystems, and yet the effect of

60   agricultural intensification on root fungal communities remains poorly understood.

61   The structure of a microbiome has substantial effects on its functioning [36].

62   However, studying the structure of a microbiome is not simple mainly due to complex

63   interrelationships among the myriad of members. Microbial co-occurrence networks can

64   unravel such relationships and offer insight into community structure [37, 38]. Network

65   analysis has been found particularly useful in recent years to understand how microbe-

66   microbe associations change in response to environmental parameters [39–42]. Network

4

67 scores can also be used to statistically identify the keystone taxa, i.e. taxa that have a large

68 influence in the community [43, 44]. Recent studies have demonstrated that such highly

69 connected taxa can explain microbiome compositional turnover better than all taxa combined

70 [45]. It has also been observed that the impact of abiotic factors and host genotypes on the

71 plant microbiome is facilitated via keystone taxa [46], and the root microbial network

72 complexity is linked to plant survival [47]. Agricultural intensification may alter the structure

73 of root microbial network and the abundance of keystone taxa, which in turn may have

74 implications for crop performance [48]. However, so far, it has not been investigated whether

75 root microbial networks differ between organic, conservation and conventional agriculture. A

76 pertinent question is whether mycorrhizal fungi that are widely regarded for their role in plant

77 productivity can also act as keystone taxa in the microbial community.

78   Here we explored the impact of farming systems on the fungal community structure

79 using the latest *PacBio SMRT sequencing* and network analysis of wheat root samples

80 collected from 60 farmlands in Switzerland. We aimed to address the following questions: a)

81 does agricultural intensity affect the structure and composition of wheat-root fungal

82 communities? b) do network complexity and the abundance of keystone taxa vary between

83 conventional, no-till and organic farming? c) which taxa act as keystone and what are the

84 drivers of such taxa in the root microbiota?

85

86 **Material and methods**

87 *Site selection and sampling*

88 Soil samples were collected in early May 2016 from wheat fields in 60 agricultural farmlands

89 in the northeast and southwest regions of Switzerland (Figure S1). Wheat fields were either

90 managed conventionally with tillage, conventionally under no-tillage, or organically under a

91 mouldboard plough tillage. Farming systems were distributed equally in both regions, and

92   each system was represented by 20 farmlands. At each farmland, 18 soil cores (4 cm

93   diameter) were collected at 0-20 cm depth with a hand auger (Figure S2). These 18 samples

94   were mixed and pooled to obtain a representative sample for a farm. The auger was cleaned

95   between sites. Five undisturbed cylindrical soil cores of 100 ml volume and 5.1 cm diameter

96   were collected for bulk density measurement and the median of the five measures was

97   considered as the estimate of bulk density for each field. Root samples were collected in June

98   2016. At each site, ten wheat plants, five per transect, were excavated using a fork spade.

99   Shoots were cut off at the height of approx. 5 cm and all roots of a specific site were pooled

100   in a plastic bag for subsequent processing. Samples were placed on ice in a cooler box for

101   transfer to the laboratory. Soil samples were processed on the same day as the collection by

102   removing plant materials, homogenizing and passing through a 2 mm sieve. Sub-samples

103   were taken for various soil physicochemical and biological analyses and stored at appropriate

104   temperature as required.

105

106   *Plant and soil analyses*

107   In the lab, roots were thoroughly cleaned under cold tap water. Subsequently, fine roots (< 1

108   mm) were cut into small pieces of about 1 cm length and thoroughly mixed. A subsample of

109   2 g of fine roots was stored in 1.5 Eppendorf tubes, lyophilized and stored at -20°C for DNA

110   extraction. The rest of the samples was used to determine AMF colonization by estimating

111   the abundance of arbuscules, hyphae or vesicles according to a modified line intersection

112   method [49] using a minimum 100 intersections per slide of two technical replicates and

113   applying a blind procedure throughout the quantification process to avoid subjectivity related

114   to the origin of the sample. Total phosphorus (P), plant available P, pH, and bulk density

115   were measured using the Swiss standard protocols (FAL, 1996). Plant available P was

116   measured according to Olsen *et al.* (1954). The abundance of AMF in soil was assessed by

117   the phospholipid fatty acid (PLFA) analysis [52]. We quantified the abundance of AMF in

118   soil by using the PLFA 16:1ω5, which is well-regarded as a biomarker for AMF because it

119   constitutes a large proportion of total PLFAs in AMF, and strong correlations between AMF

120   abundance in the soil and concentrations of the PLFA 16:1ω5 have been observed previously

121   [53].

122

123   *DNA extraction and SMRT sequencing*

124   For each sample, 200 mg of roots (dry weight) was used for DNA extraction using 600 mL of

125   Nucleo spin lysis buffer PL1 for 15 min at 65 °C followed by the NucleoSpin Plant II kit

126   (Macherey & Nagel, Düren, Germany). The DNA samples were amplified with the primer

127   pair *ITS1F-ITS4* [54, 55] targeting the entire ITS region (approx. 630 bp) [56]. The forward

128   and reverse primers were synthesized with a 5-nucleotide-long padding sequence followed by

129   barcode tags at the 5' end to allow multiplexing of samples within a single sequencing run

130   [57]. Library preparation and SMRT sequencing were conducted at the Functional Genomics

131   Centre Zurich (http://www. fgcz.ch) on the PacBio® RS II Instrument (PacBio, San Diego,

132   CA, USA). Details of PCR conditions and sequence data processing are described in the

133   *Supplementary Information*. In brief, the SMRT Portal was used to extract the circular

134   consensus sequences (CCS) from the raw data (available from the European Nucleotide

135   Archive, study accession number: PRJEB27781). The CCS of at least five passes yield

136   similar error rates as 454 or MiSeq sequencing platforms [56, 57]. The CCS reads were

137   quality filtered in Mothur (v.1.35.0) [58]. Quality reads were demultiplexed based on the

138   barcode-primer sequences using *flexbar* [59]. *De novo* chimera detection was performed on

139   quality reads using UCHIME [60]. To avoid unwanted multi-primer artefacts, we deleted

140   reads where full-length sequencing primer was detected within the read [61]. We clustered

141   the quality sequences into operational taxonomic units (OTUs) at ≥ 98% sequence similarity

142   with the UPARSE series of scripts [62]. Reads were de-replicated, and single-count and

143   chimeric sequences were excluded for OTU delineation. The OTUs of low abundance (less

144   than 0.1% global abundance and less than 0.5% abundance within a specific sample) were

145   removed from the dataset (Figure S3). On average 357 OTUs were found per site and a total

146   of 823 OTUs for all 60 sites. The OTUs were classified taxonomically against the UNITE

147   database [63]. The OTU and taxonomy tables were filtered to exclude OTUs classified as

148   non-fungal.

149

150   *Statistical analyses*

151   Alpha diversity indices such as OTU richness, Sheldon evenness and Shannon-Weaver index

152   were calculated from the rarefied fungal OTU table using the *phyloseq* package [64] in R

153   v3.4 [65]. The effect of farming systems and wheat varieties on fungal community structure

154   was assessed by performing PERMANOVA and canonical analysis of principal coordinates

155   (CAP) with 999 permutations in PRIMER-E (PRIMER-E, Plymouth, UK). Fungal beta

156   diversity patterns were only assessed on OTUs that were present in at least two samples.

157   Homogeneity of multivariate dispersions was checked with the PERMDISP test using the

158   Bray-Curtis similarity matrix in PRIMER. We also identified the indicator taxa for each

159   farming system using the *indicspecies* package in R [66]. Co-occurrence patterns in fungal

160   communities were assessed by performing network analysis using the maximal information

161   coefficient (MIC) scores in MINE statistics [67]. MIC is an insightful score that reveals

162   positive, negative and non-linear associations among OTUs. To minimize pairwise

163   comparisons, network analysis was performed on OTUs that were present in at least two

164   samples, resulting in 826 fungal OTUs. The overall meta-network was constructed with 60

165   samples whereas the three farming specific networks were constructed with 20 samples each.

166   The MIC associations were corrected for false discovery rate (FDR) [68] and the final

167   networks were constructed with relationships that were statistically significant (P<0.05) after

168   FDR correction. The networks were then visualized in Cytoscape version 3.4.0 [69]. The

169   *NetworkAnalyzer* tool was used to calculate network topology parameters. We also evaluated

170   networks against their randomized versions using the Barabasi-Albert model available in

171   *Randomnetworks* plugin in Cytoscape v2.6.1. The structural attributes of fungal networks

172   such as degree distribution, mean shortest path, clustering coefficient were different from

173   random networks with an equal number of nodes and edges. The OTUs with the highest

174   degree and highest closeness centrality, and the lowest betweenness centrality scores were

175   considered as the keystone taxa [43]. We calculated the influence of various taxa in network

176   stability by dividing the number of nodes belonging to a particular taxon by the number of

177   connections (edges) it shared. Finally, we performed Random Forest Analysis [70] to explore

178   the determinants of the identified keystone taxa with 999 permutations using the

179   *randomforest* and *rfPermute* packages in R [71]. The best predictors were identified based on

180   their importance using the *importance* and *varImpPlot* functions. Increase in node purity and

181   mean squared error values were used to calculate the significance of the predictors using the

182   *randomForestExplainer* package [72]. The factors significant at P<0.01 were selected as the

183   predictors of keystone taxa. Agricultural intensity index was calculated according to a

184   previous study [73] based on the information collected from farmers (Büchi et al., submitted).

185   Agricultural intensity index was estimated using the information on three anthropogenic input

186   factors: fertilizer use, pesticide use and the consumption of fuel for agricultural machineries.

187   These factors were also included for assessing agricultural intensity in a previous study [74].

188

189   **Results**

190   *Overall structure and co-occurrence*

9

191 Farming systems significantly influenced the root fungal community structure with three

192 distinct clusters for organic, conventional and no-tillage fields (Figure 1A). A

193 PERMANOVA test confirmed the significant effect of farming systems (pseudo F = 1.42;

194 P<0.05). However, alpha diversity indices and the overall taxonomic composition did not

195 vary between the conventional, no-till and organic systems (Figure S4, S5). A non-significant

196 PERMDISP test (F = 2.072; P = 0.202) indicated homogenous dispersions of samples across

197 systems. Further, a pairwise comparison in PERMDISP revealed that there was no significant

198 difference in dispersions between organic and conventional (F = 1.068; P = 0.372), and

199 organic and no-till (F = 0.870; P = 0.435). We found no impact of wheat varieties on

200 community structure and this was reinforced by a non-significant PERMANOVA test

201 (Pseudo F = 0.972; P = 0.595) (Figure S6). However, geographical locations i.e., northeast

202 and southwest regions had an impact on root fungal community structure (Figure S7).

203 Indicator species analysis was performed to test which taxa are characteristic for each of the

204 three farming systems. Root inhabiting *Trichoderma*, a member of *Hypocreales*, was the only

205 indicator taxon for conventional farming system whereas seven fungal taxa (e.g.,

206 *Cyphellophora, Myrmecridium, Phaeosphaeria, Cadophora, Pyrenochaeta, Solicoccozyma*,

207 and *Conocybe*) were the indicator taxa for no-till farming (Table S1). Six taxa of *Sordariales,*

208 *Cantharellales*, and *Agaricales* were indicator taxa for organic farming with *Chaetomium* and

209 *Psathyrella* as the only known genera.

210   The overall network of root fungal communities in 60 samples revealed distinct co-

211 occurrence patterns (Figure 1B). The meta-network consisted of 378 nodes and 1602

212 significant (P<0.05) edges. This network with strong power-law distribution of degrees had a

213 diameter of 8, average number of neighbours of 8.476, and a clustering coefficient of 0.258.

214 For the overall network, the top ten taxa with the highest degree, highest closeness centrality,

215 and lowest betweenness centrality were selected as the keystone taxa (Table S2). Seven of

10

216   these taxa belonged to arbuscular mycorrhizal orders *Glomerales, Paraglomerales*, and

217   *Diversisporales* and the remaining three belonged to *Tremellales*. Indeed, the majority of the

218   associations were from these four orders with *Glomerales* forming the largest guild with the

219   maximum number of nodes and associations in the network. Overall, farming systems

220   significantly affected fungal community structure with mycorrhizal orders playing a major

221   role in the network complexity.

222

223   *Farming specific co-occurrence networks*

224   Owing to the significant difference in fungal community structure across three farming

225   systems, we further evaluated root fungal networks for each farming system separately. The

226   networks displayed remarkable differences in their structure and topology (Figure 2). The

227   network of conventional farming consisted of 261 nodes (e.g., taxa) and 315 edges

228   (associations between taxa) while the no-till network consisted of 267 nodes and 341 edges.

229   In stark contrast, the organic farming network consisted of 301 nodes and 643 edges. The

230   average number of neighbours and the clustering coefficient of the organic farming network

231   were also considerably higher than for the other two networks (Figure 2). The higher

232   complexity and connectivity in the organic farming network were supported by the

233   abundance of keystone taxa, taxa that are central to community structure. The organic

234   farming network harboured 27 of such keystone taxa compared to two in the no-till network

235   and none in the conventional one (Figure 2; Table S3). The majority of these keystone taxa

236   belonged to the orders *Glomerales*, *Tremellales* and *Diversisporales* with a noticeable

237   presence of taxa from the orders *Paraglomerales*, *Sebacinales* and *Hypocreales*.

238   Higher connectivity in the organic farming network was visible in the distribution of

239   degrees, which indicates the number of associations shared by each node in a network (Figure

240   3). The organic farming network had a much stronger power-law distribution than the

11

241   conventional and no-till ones, despite the similar node distribution across root fungal orders

242   (Figure S8). We calculated the proportional influence of various orders in the microbiota by

243   dividing the number of nodes belonging to a particular order by the number of connections

244   (edges) it shared. It revealed the orders that exhibited maximum connections across three

245   farming systems and thereby influence the network structure. Various orders exhibited

246   considerable differences in their proportional influence in the stability of root microbiota.

247   Orders such as *Sordariales* and *Agaricales* showed a major influence in the conventional

248   network structure, and *Sordariales, Cantharellales* and *Mortierellales* in the no-till network.

249   In addition to *Tremellales and Hypocreales*, three mycorrhizal orders *Glomerales*,

250   *Paraglomerales* and *Diversisporales* showed a major influence on network stability under

251   organic farming. Overall, the organic farming network formed a much more complex

252   network and harboured more keystone taxa than the other two farming networks.

253

254   *Drivers of keystone taxa*

255   Random forest analysis revealed that soil phosphorus content, bulk density, pH, and

256   mycorrhizal colonization best explained ($P<0.01$) the occurrence of keystone taxa (Figure 4).

257   Most of these parameters were also significantly ($P<0.05$) correlated with the alpha-diversity

258   indices, indicating their importance for the overall root fungal communities (Table S4). The

259   majority of keystone taxa belonged to mycorrhizal orders, and mycorrhizal colonization of

260   wheat roots was significantly ($P<0.01$) higher in the organic fields than in the conventional

261   and no-till fields (Figure S9). Consistent with this, the abundance of mycorrhizal PLFA in

262   soil were also significantly ($P<0.01$) higher in the organic fields. Agricultural intensity had a

263   significantly negative impact on mycorrhizal colonization in roots and the abundance in soils

264   (Figure 4). Agricultural intensity was significantly ($P<0.05$) different across three farming

265   systems with conventional as the most intensive and organic as the least intensive system

12

266   (Figure S9). This trend was opposite for the abundance of keystone taxa i.e., the number of

267   keystone taxa was 27 in the organic farming network, 2 in the no-till, and 0 in the

268   conventional network. Taken together, the root fungal network complexity, abundance of

269   keystone taxa and mycorrhizal abundance showed an opposite trend to that of agricultural

270   intensification across farming systems.

271

272   **Discussion**

273   It is now well established that root-associated microbiota plays an important role in plant

274   diversity, community composition and performance [24, 32, 75]. Consequently, it is

275   important to understand how microbial communities harboured in crop roots are affected by

276   farming systems and how key microbial players can be harnessed for ecological

277   intensification of agroecosystems. However, with much of the previous work only focussing

278   on the soil microbiota, our understanding of the effects of farming systems on root-associated

279   microbiota is still rudimentary. Moreover, previous studies mostly focused on microbial

280   alpha- and beta diversity patterns and the impact of different farming systems on microbial

281   network structure is poorly understood.

282   Our results showed that agricultural intensity was the highest under conventional

283   farming and the lowest under organic farming. The overall structure of root microbiota was

284   significantly influenced by farming systems. This is also consistent with studies on soil

285   microbiome where a large number of reports showed a significant impact of farming systems

286   [20–22, 76, 77]. For the root microbiota, Hartman *et al.* (2018) observed in a farming

287   experiment that root fungal communities were only affected by tillage intensity and not by

288   conventional or organic farming. It should be noted that most of these studies investigated

289   microbial communities in field-trials [20–22, 30, 77]. While a major strength of field-trials is

290   that farming treatments are imposed under a homogenous management and at one location

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291   with a specific soil type, management effects on microbial patterns may be different in actual

292   farmlands and thus, the results obtained at one location cannot be generalized. It is necessary

293   to investigate whether microbial community characteristics observed in field-trials can be

294   generalized in on-farm research and across many fields. To our knowledge, this is the first

295   on-farm study on root fungal communities under different farming systems focussing on

296   conventional, organic and conservation agriculture. Our results show that wheat roots under

297   different farming systems harbour distinct fungal communities and with varying network

298   complexity.

299   Microorganisms do not thrive in isolation and rather form complex association

300   networks. Such networks hold special importance for gaining insight into microbiome

301   structure and its response to environmental factors [37, 38, 46]. Our study highlights how

302   farming systems impact the network structure of root microbiota, and uncovers, for the first

303   time, that organic farming harbours a significantly more complex network than the

304   conventional and no-till farming. The organic network exhibited a much stronger power-law

305   distribution of degrees with many highly connected nodes whereas no-till and conventional

306   networks were dominated by less connected peripheral nodes. It has been shown that

307   complex networks with greater connectivity are more robust to environmental perturbations

308   than simple networks with lower connectivity [78]. In this sense, the higher complexity of

309   organic networks may indicate that the root microbiota under organic management is more

310   resilient to environmental stresses as different taxa can complement each other and network

311   complexity may provide an insurance for network stability if specific taxa go extinct.

312   However, further studies are necessary to corroborate this observation.

313   Keystone taxa are the highly connected taxa that play important roles in the

314   microbiome and their removal can cause significant changes in microbiome composition and

315   functioning [43, 44]. Although previous studies have reported keystone taxa in various

316   environments [40, 79, 80], reports on keystone taxa in the root microbiome are very limited.

317   The organic farming network exhibited by far the highest connectivity and comprised most of

318   the keystone taxa. It should be noted that fungal richness did not vary significantly between

319   the farming systems nor did the node distribution, and yet we observed a clear difference in

320   the network structure. Nodes in the organic network shared more associations and there were

321   also many highly associated keystone taxa. The abundance of these keystone taxa did not

322   vary between the three farming systems but these taxa shared considerably more associations

323   in the organic farming (Figure S10). A previous study reported a gradient of root fungal

324   assemblages from conventional to organic to natural grasslands, i.e., root microbiota in the

325   organic farming was more developed than the conventional farming [81]. These observations

326   indicate a possibility that microbiome complexity is not necessarily determined by the

327   number of taxa in the community, but rather the number of associations that those taxa share

328   amongst them.

329   Remarkably, the majority of these keystone taxa were arbuscular mycorrhizal fungi

330   (AMF) belonging to the orders *Diversisporales, Glomerales*, and *Paraglomerales*. The

331   symbiotic association of AMF that started more than 400 million years ago is formed by

332   ~80% of terrestrial plants [33, 82]. The observation that AMF can enhance plant productivity

333   [83] make them a crucial player in agroecosystems. The importance of AMF for the root-

334   associated microbiota, particularly under organic farming, is congruent with the higher

335   abundance of AMF in roots and soils observed in the organic farmlands in this study (Figure

336   S9). Mycorrhizal root colonization was significantly higher under organic farming than non-

337   till and conventional farming systems. This pattern was also evident for mycorrhizal

338   abundance in the soil as measured by AMF-specific PLFA concentration. While previous

339   studies also found significantly higher AMF abundance and diversity in organic farmlands

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340   than in the conventional ones [81, 84], the pivotal role of AMF for the entire root-associated

341   microbiota in agroecosystems is reported here for the first time.

342   One of the non-mycorrhizal keystone taxa in the organic farming belonged to the

343   order *Sebacinales*. Members of this order are highly diverse root endophytes and are thought

344   to form neutral and beneficial interactions with plants [85]. Our observation of *Sebacinales* as

345   keystone taxa is consistent with a previous report that found a consistently higher abundance

346   of *Sebacinales* in organic farmlands [29]. Since keystone taxa are linked to network

347   complexity, beneficial endophytic keystone taxa such as AMF and *Sebacinales* may enhance

348   the network complexity and thereby the stability of the root microbiome. Several other

349   keystone taxa in the overall and organic networks belonged to the order *Tremellales*. This

350   widespread group of Basidiomycetes contains many yeast species and have been reported in

351   plant roots in temperate regions [86]. Members of this fungal order were also recently found

352   as keystone taxa in the root microbiome across eight forest ecosystems in Japanese

353   Archipelago [48]. Interestingly, we found that two of the keystone taxa (OTU_10, OTU_11)

354   were members of the *Dioszegia* genus that was also found as keystone by Agler *et al.* (2016).

355   It was shown that the effect of abiotic factors on microbiome was mediated via *Dioszegia* in

356   *Arabidopsis thaliana*. The consistent identification of *Dioszegia* as a keystone taxon across

357   studies suggests its importance and highlights a potential that it can be harnessed for

358   manipulation of the plant microbiome. Future studies are now needed to specifically

359   manipulate this taxon to test how it influences microbiome composition and functioning.

360   There were no common fungal groups between indicator taxa and keystone taxa. It should be

361   noted that indicator taxa are identified based on their higher/lower abundance under a

362   particular farming system whereas keystone taxa are identified using a comprehensive

363   algorithm that focuses on the number of associations a taxon shares and its position in the

16

364   microbiome. Thus, indicator taxa and keystone taxa highlight different aspects of microbial

365   communities.

366   Beyond statistical identification of keystone taxa, this study also revealed the factors

367   driving their abundance in the wheat root microbiota. Bulk density plays a pivotal role in root

368   elongation rates, rooting density and root architecture [87], which have significant

369   implications for microbial recruitment into the roots. We found soil phosphorus levels and

370   plant available phosphorus were the strongest determinants of keystone taxa. The majority of

371   keystone taxa were mycorrhizal in nature, and phosphorus is well-acknowledged for its

372   importance for mycorrhizal associations [88]. Similarly, soil pH is a known driver of fungal

373   communities, especially, mycorrhizal fungi [89, 90]. Thus, the identification of soil

374   phosphorus levels, pH and bulk density as the determinants of keystone taxa in root

375   microbiota is plausible. The number of keystone taxa was the highest under organic farming

376   where agricultural intensity was the lowest and conversely, intensification was the highest

377   under conventional farming where root microbiota did not have any keystone taxa. Thus,

378   agricultural intensification might have negatively affected the network complexity and

379   keystone abundance in the root microbiota. It is important to mention that identification of

380   keystone taxa are based on the analysis of correlations (associations) among taxa, and further

381   research is necessary to show causality, in terms of the impact of keystone taxa on

382   microbiome structure and functioning.

383

384   **Conclusions**

385   The structure and composition of root microbiota play an important role in agroecosystems

386   and yet there is a significant dearth of knowledge about the effect of agricultural

387   intensification on the root microbiota. Our study shows that the microbiome complexity and

388   the abundance of keystone taxa were the highest under organic farming where agricultural

17

389   intensity was the lowest. The higher co-occurrence of members of microbial communities

390   under the organic farming can be indicative of greater ecological balance and stability of the

391   microbiome. A key strength of this study is that the samples were collected from 60 fields

392   and the reported effects can be generalized because samples were taken from an extensive

393   range of fields at different locations with different management regimes. The recent concept

394   of smart farming (*sensu* Wolfert *et al.*, 2017) emphasizes *thinking outside the box*. The

395   potential for harnessing plant microbiome for sustainable agriculture was also highlighted

396   recently [92]. Mycorrhizal fungi are well-regarded for their effects on plant productivity and

397   thus, mycorrhizal keystone taxa may be targeted as a tool for smart farming.

398

Acknowledgement

Author contributions

407   MvdH, RC and TK conceived and designed the study; FW, LC and MM conducted the

408   sampling; AYH and MM performed molecular and mycorrhizal analyses; SB performed

409   statistical analyses and wrote the manuscript; FW also contributed to manuscript preparation;

410   all authors edited the manuscript.

Sequence availability

412   All sequences generated in this study are available in the European Nucleotide Archive under

413   the study accession number: PRJEB27781.

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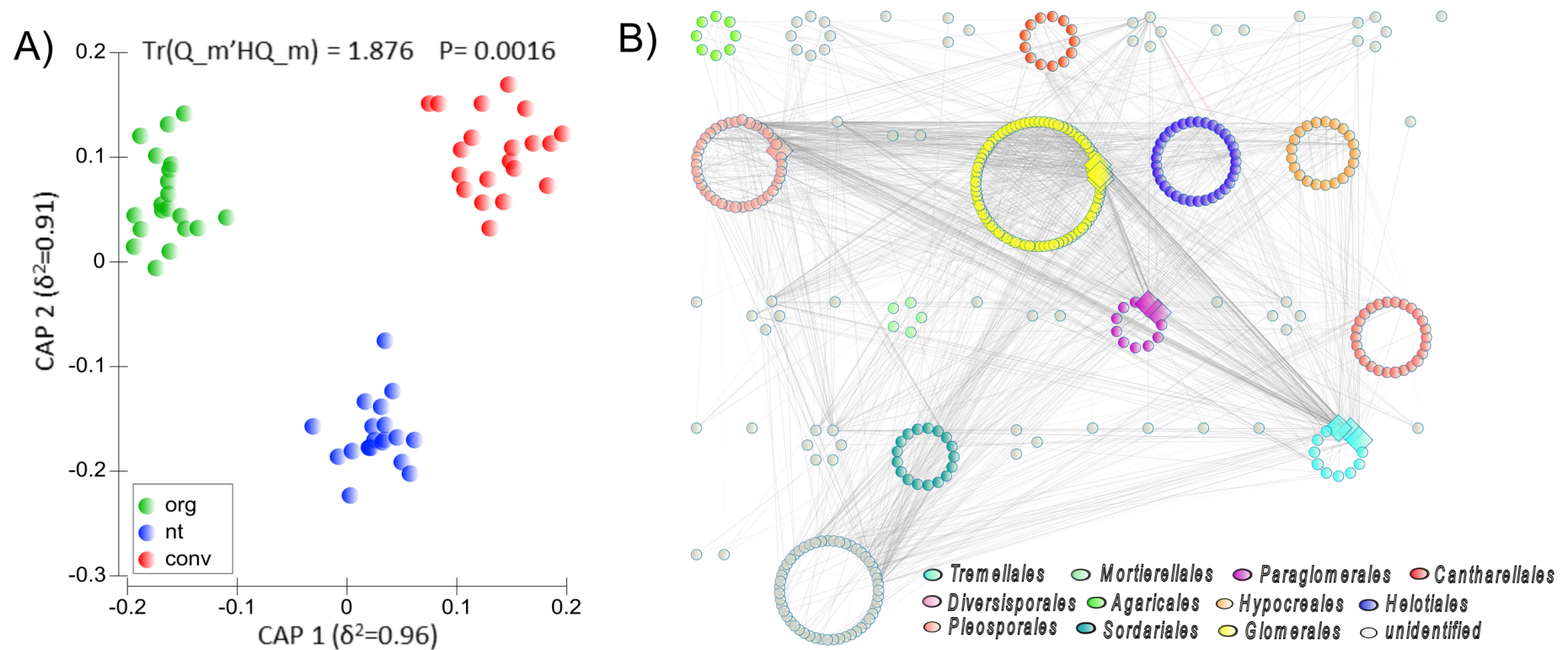
Figure 1. A) Canonical analysis of principal coordinates (CAP) revealing a significant impact of farming systems on fungal community structure. **B)** The overall network of root fungal communities across three farming systems. The overall network is arranged according to orders. White, red and wavy lines represent positive, negative and non-linear relationships, respectively. Large diamond nodes indicate the keystone taxa in the network. Top ten nodes with the highest degree, highest closeness centrality, and lowest betweenness centrality were selected as the keystone taxa [43]. Out of the ten keystone taxa in the overall network, seven belonged to mycorrhizal orders, *Glomerales, Paraglomerales*, and *Diversisporales*.
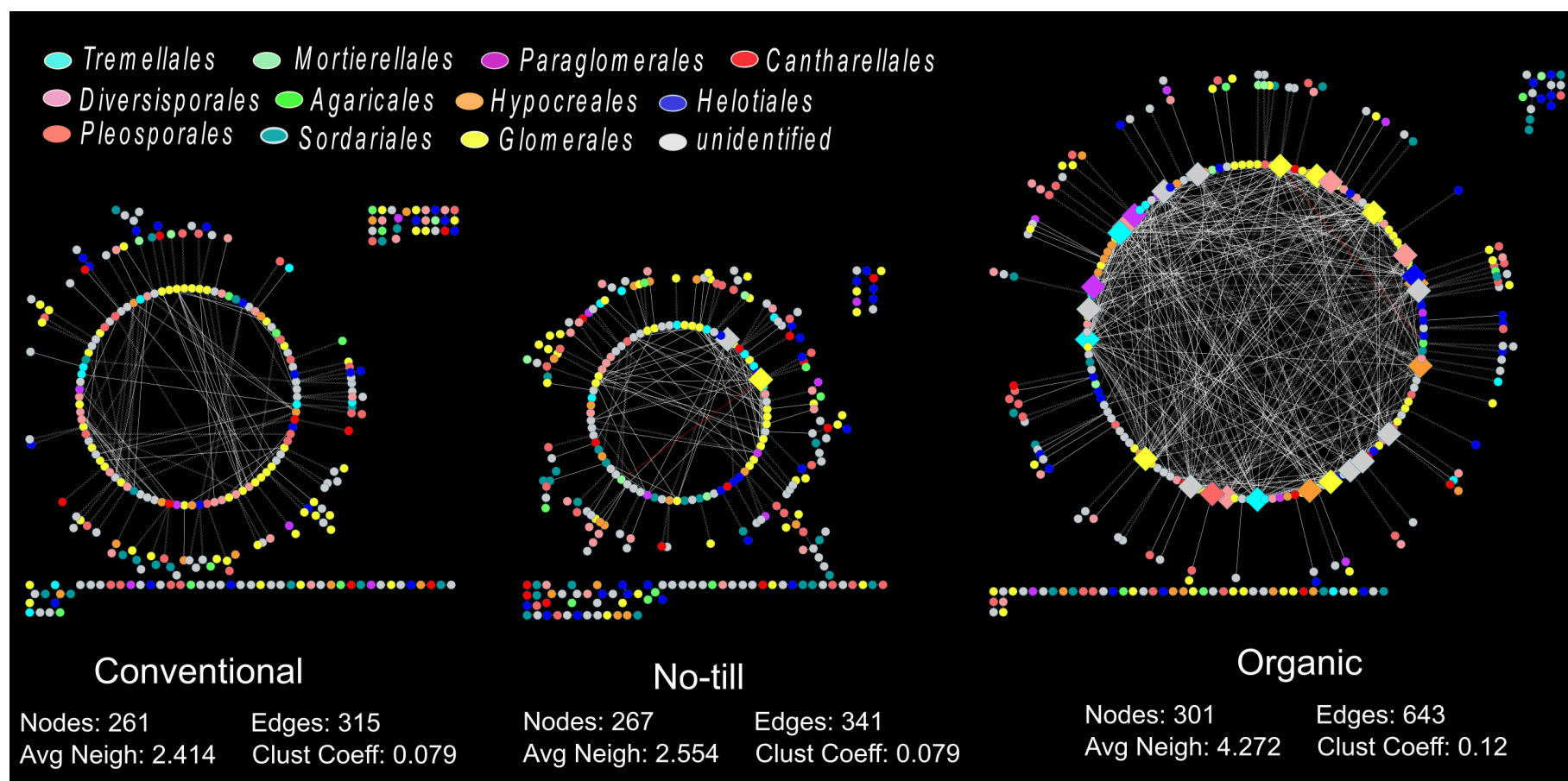
Figure 2. Farming system specific root fungal networks. Each network was generated with root samples from 20 farmlands belonging to that farming systems. Large diamond nodes indicate the keystone taxa whereas circular nodes indicate other taxa in the network. White, red and wavy lines represent positive, negative and non-linear relationships, respectively. Despite having similar number of nodes, the organic network displayed twice more edges and many highly connected nodes than no-till and conventional networks that were dominated by less connected peripheral nodes.
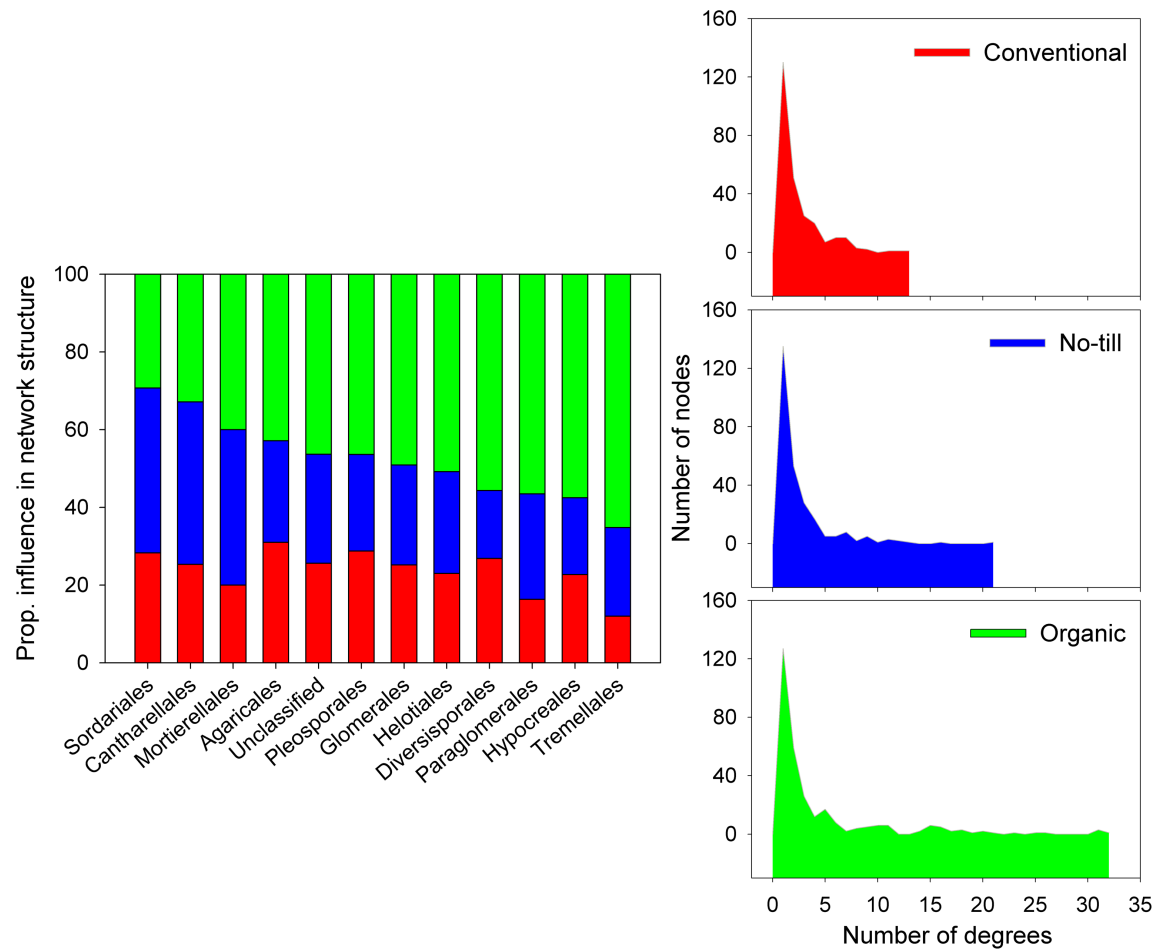
25

Figure 3. Proportional influence of various fungal orders in affecting the stability of root microbiota (left panel). The influence was calculated by diving the number of nodes belonging to a particular fungal order by the number of connections (edges) it shared. It illustrates the orders that exhibit maximum connections across farming systems and thus influences network structure most. Distribution of degrees in three farming systems (right panel with three plots). Degree indicates the number of associations shared by each node in a network. In conventional, farming, the number of degrees was limited to a maximum of 12 compared to the no-till network that had a maximum of 22 degrees. On the other hand, organic farming had many nodes with over 20 degrees.
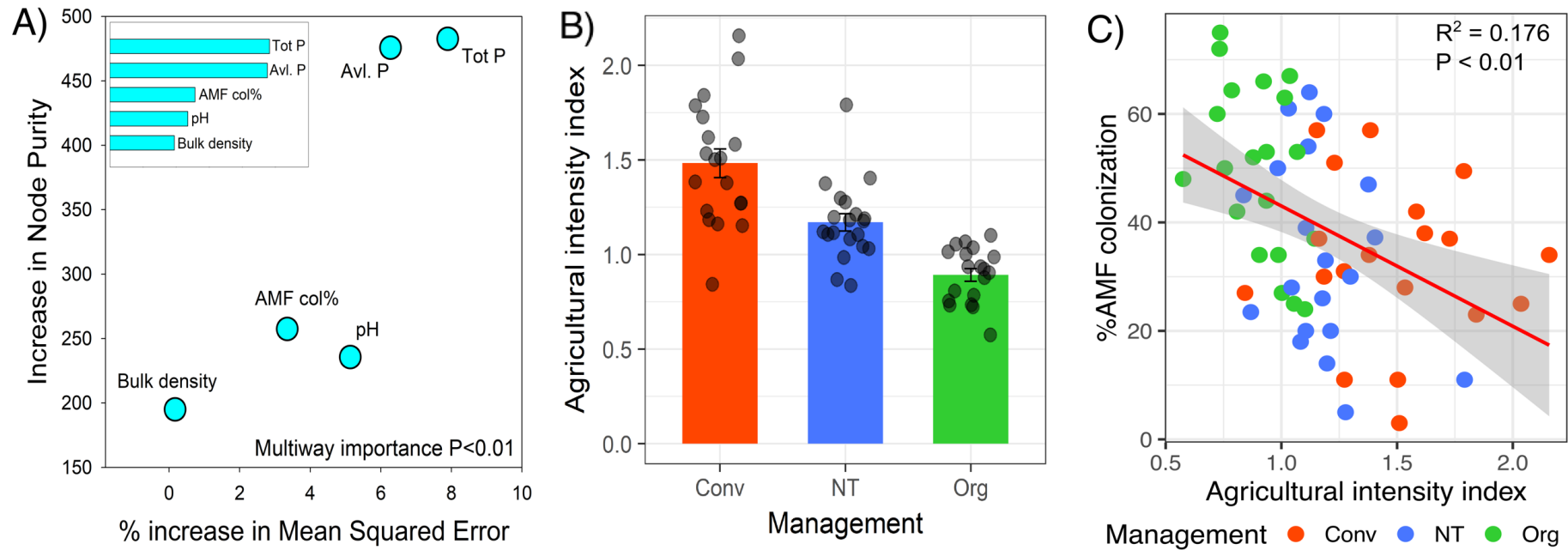
Figure 4. A) Results of Random Forest Analysis showing the relative contribution of various factors in determining the abundance of keystone taxa. The mean squared error (MSE) indicates the prediction accuracy of each factor. The top (P<0.01) five drivers were total phosphorus, plant available phosphorus (Olsen P), AMF root colonization, pH and bulk density. B) Agricultural intensity index across three farming systems. Agricultural intensity index was estimated using the information on three anthropogenic input factors: fertilizer use, pesticide use and the consumption of fuel for agricultural machineries. C) Relationship between agricultural intensity and mycorrhizal root colonization. Agricultural intensification had a significantly (P<0.01) negative impact on the root colonization of AMF. Agricultural intensity was the highest under the conventional farming and the lowest under the organic farming, which was opposite for the AMF colonization.