- Soil properties and food webs showed large differences between crop types
- Soil food webs were generally degraded, but olive groves were better preserved than vineyards
- Microfaunal food webs differed little between organic and conventional fields
- In semi-arid conditions, organic farming induced little soil improvement
- Active soil conservation practices are required to increase soil quality

ORGANIC   CONVENTIONAL

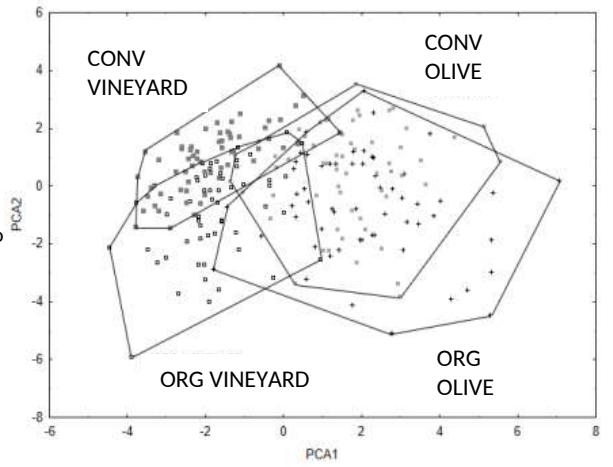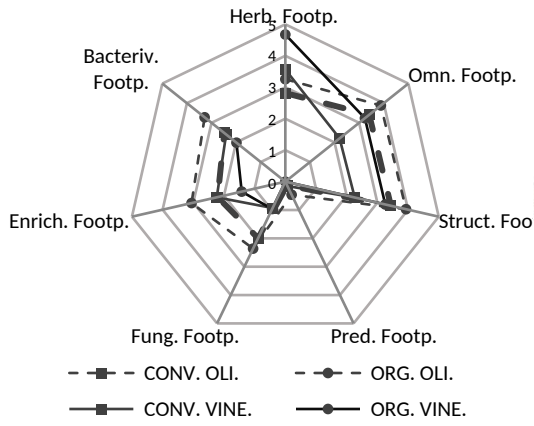SOIL NEMATODE DIVERSITY

Herb. Footp.
Bacteriv. Footp.
Omn. Footp.
Enrich. Footp.
Struct. Foo
Fung. Footp.
Pred. Footp.

– ■ – CONV. OLI.   – ● – ORG. OLI.
—■— CONV. VINE.   —●— ORG. VINE.

CONV VINEYARD   CONV OLIVE

ORG VINEYARD   ORG OLIVE

PCA2
PCA1

1   Microfaunal soil food webs in Mediterranean semi-arid agroecosystems. Does organic

2   management improve soil health?

3   Sara Sánchez-Moreno[1*], Miriam Cano[1], José Antonio López-Pérez[2], José M. Rey Benayas[3].

4   [1]UPF (DTEVPF) National Institute for Agriculture and Food Research and Technology, Ctra. de la

5   Coruña km 7.5 28040 Madrid, Spain. [2] Centro de Investigación Apícola y Agroalimentario

6   (CIAPA, IRIAF) 19180 Marchamalo, Guadalajara, Spain. [3]Forest Ecology and Restoration Group,

7   Departamento de Ciencias de la Vida, Edificio de Ciencias, Universidad de Alcalá, 28805 Alcalá

8   de Henares, Spain. *Corresponding author. Tel.: +34 913476956. E-mail address:

9   sarasm@inia.es (S. Sánchez-Moreno).

10

11   ABSTRACT

12   Soil food webs, which are responsible for relevant ecological functions in agroecosystems such

13   as nutrient cycling and pest and disease suppression, represent a crucial aspect of agricultural

14   sustainability. We studied soil properties and microfaunal food web diversity and functioning

15   in six paired organic and conventional fields located in Central Spain to assess the effects of

16   organic farming on soil diversity and functioning in semi-arid conditions. We hypothesized that

17   organic farming may enhance functioning of soil food webs. Our results showed larger

18   differences between crop types, namely olive groves and vineyards, than between farming

19   scheme, i.e. organic and conventional fields, and few benefits of organic farming in terms of

20   soil fertility. Soil properties (total N, C, and P, available P and K, electrical conductivity, NH_4^+,

21   NO_3^-, soil moisture, pH) tended to present higher values in vineyards than in olive groves and

22   in conventional than in organic fields. Some plant-parasitic nematodes were associated to

23   organic fields, especially in vineyards, and all soils fell within a degraded soil food web

24   condition, with low Structure and Enrichment Index values. Nematode metabolic footprints

25 showed relevant seasonal dynamics, with the more intensive herbivore activity in spring. We

26 conclude that the lack of conventional pesticides and mineral fertilizers is probably not enough

27 to improve soil conservation in semi-arid Mediterranean agroecosystems, and thus active soil

28 conservation practices, as reduced tillage or cover cropping, are required to increase

29 agroecosystem sustainability.

30

1. Introduction

32 Microfaunal food webs play critical role in soil functioning. Nematodes, the most abundant

33 metazoan organisms in microfaunal food webs, are relevant components of belowground C

34 cycling in spite of their low absolute biomass (Pausch et al., 2016). They occupy multiple

35 positions in the soil food web and present high taxonomic and functional diversity (Ettema,

36 1998). Among other functions, nematodes participate in nitrogen and carbon mineralization

37 (Bouwman et al., 1994), the regulation of microbial communities (Villenave et al., 2004), pest

38 suppression (Steel and Ferris, 2016), and the redistribution of other organisms in the soil

39 matrix (Knox et al., 2003). Appropriate soil management to enhance soil fauna contribution to

40 soil functioning and derived ecological services should be promoted to increase agricultural

41 sustainability.

42 Different management systems intended to reduce soil chemical and physical disturbance may

43 improve such agricultural sustainability. In this context, organic farming has been under

44 continuous expansion during the last years in the European Union. The area under organic

45 farming in Europe has notably increased, with the mean annual growth from 6% (EU-27) to

46 13% (EU-12) from 2002 to 2013 (European Commission, 2013). In 2015, the eight countries

47 with largest areas under organic management hold between 0.46 and 1.9 x 10^6 ha per country.

48 Among those Spain demonstrates the largest share of and is followed by Italy and France

49   (EUROSTAT, 2016a). Nowadays, public concerns on the effects of pesticides on human health

50   and the environment have increased (Miller, 2013).

51   Several local studies and meta-analyses have shown the value of organic farming for

52   biodiversity. In their meta-analyses, Bengtsson et al. (2005) and Hole et al. (2005) found that a

53   number of taxonomic groups (including birds, insects, mammals, and plants) usually show

54   increased species richness and abundance in organic as compared to conventional farming

55   systems. Although the effects of organic farming on below-ground diversity has been less

56   studied, some reports indicate that organic farming increases arbuscular-mycorrhizal fungi

57   (Verbruggen et al., 2010) and supports higher microbial activity (França et al., 2007). Other

58   studies, however, have found organic farming to be more effective conserving aboveground

59   than belowground diversity (Flohre et al., 2011). A comparison of organic and conventional

60   kiwifruit orchards showed that earthworms were negatively affected by conventional

61   practices, while mesofauna (enchytraeids, mites, and collembolans) were stimulated by

62   conventional management (Castro et al., 2015), but reports on the effects of organic farming

63   on soil diversity under semiarid conditions are scarce. Under semiarid conditions, organic

64   farming may increase C and N soil pools (Parras-Alcántara et al., 2015), and conversion from

65   grassland to cultivated organic farming might increase functional microbial diversity (Nautiyal

66   et al., 2010).

67   Vineyards and olive orchards are typical semi-arid Mediterranean crops and a major feature of

68   the heritage in the Mediterranean basin, where they play an important environmental role

69   fixing soils, maintaining biodiversity, and contributing to producing environmentally rich

70   landscapes (Biasi et al., 2012). There are generally few studies on soil diversity in these woody

71   crops, and the effects of different management systems in such diversity and their associated

72   functions and services have been little addressed.

73   Among soil organisms, nematodes possess several attributes that make them good indicators.

74   Nematodes are abundant, ubiquitous and diverse, participate in several soil food web links and

75   are sensitive to agricultural disturbance (Yeates, 2003). Due to such attributes, multiple

76   nematode-based indicators have been developed. Besides classical diversity indices, maturity

77   indices have been used to infer the position of the nematode community along the ecological

78   succession (Bongers, 1990; Korthals et al., 1996), and soil food web indices are used to infer

79   food web complexity and main channels of organic matter decomposition (Ferris et al., 2004).

80   Nematode functional guilds have been shown to reflect soil food web functions in response to,

81   for example, global change (Cesarz et al., 2015). Nematode metabolic footprints (NMF),

82   proposed by Ferris (2010), assess the magnitude of nematode contribution to soil functioning

83   by partitioning the amount of C used by nematodes in production (biomass growth, egg laying)

84   and lost in respiration. The inference of soil nematode biomass and the partitioning of

85   nematode C use into such components allow inferring nematode trophic group activity

86   (herbivores, bacterivores, fungivores, and omnivore/carnivore nematodes) and functional

87   groups (enrichment, basal, and structure nematode indicators). NMF has been found to be

88   sensitive to tillage (Zhang et al., 2012), cover cropping (Ferris et al., 2012b), fertilization (Zhang

89   et al., 2016a), and microclimate variations (Bhusal et al., 2015). The inclusion of inferred

90   nematode biomass in calculation of nematode-based diversity indices (Ferris and Tuomisto,

91   2015) open new perspectives in the analyses of the functionality of soil organisms.

92   Spain holds the largest vineyard and olive-growing areas in Europe, with 0.8 and 2.2 x10^6 ha,

93   respectively (EUROSTAT, 2016b). Within the country, Castilla-La Mancha, in the South-Central

94   part of Spain, possesses the largest area of vineyards and the second largest area of olive trees

95   (MAPAMA, 2017). Here we studied soil properties and nematode food webs in organic and

96   conventional olive groves and vineyards in South-Central Spain to evaluate 1) nematode

97   diversity in woody crops under semiarid conditions, and 2) the effects of organic and

98   conventional practices on soil functioning in such systems. We hypothesized that organic

99   agroecosystems harbour a greater nematode diversity, soil food web complexity, and soil

100   functioning that conventional agroecosystems.

101

102   **2.   Material and methods**

103

104   *2.1. Study site*

105   The study was carried out in Ciudad Real province (near Valdepeñas, 38° 45' N - 3° 23' W,

106   Castilla-La Mancha region, South-Central Spain). The area has a typical Mediterranean climate

107   with a mean annual temperature of 15.6 °C and precipitation of 418 mm (MAPAMA, 2017b).

108   Soils included in the study were classified as calcil cambisols and presented   a loam texture

109   with an average of 20.8% clay, 31.7% silt and 47.5% sand.

110   Three olive growing and three vine growing sites were chosen in the study area. At each site,

111   two adjacent fields, one conventional and one organic, were selected as representatives of

112   typical management in the region. If no adjacent fields were available, the organic and

113   conventional fields at each pair were as nearby as possible (four out of the six pairs were

114   adjacent or a few meters apart, for the other two maximum distance between paired fields

115   was 500 m). In total, 12 fields (3 organic and 3 conventional vineyards and 3 organic and 3

116   conventional olive groves) were included in this study. Each pair of fields was selected to be as

117   similar as possible to make straightforward comparisons. Location, type of management, field

118   area, tree density (in the case of olive groves), type of training (in vineyards), and irrigation

119   systems in the fields are indicated in Table 1.

120   Field management was quite similar among conventional or organic fields. All conventional

121   fields were fertilized in spring with mineral fertilizers (NPK 15:15:15), at a rate of 450 kg/ha in

122   vineyards and 2 kg/tree in olive groves. Chemical weeding with glyphosate at standard

123 recommended field doses occurred once a year (January-February) in olive groves and twice a

124 year (July and August) in vineyards. In conventional vineyards, paclobutrazol was applied

125 annually as plant growth regulator. Tebuconazole and copper oxychloride were used a

126 maximum of once a year in vineyards and olive groves, respectively.

127 Organic fields were all certified and did not use any mineral fertilizer or chemical pesticide.

128 Sulphur 98.5% was typically used every spring as main fungicide in organic vineyards. In

129 organic olive groves *Bacillus thuringiensis* was used to control insect pests when necessary. .

130 One organic olive grove grew and incorporated a chickpea green manure every three years.

131 Sheep manure was used as soil amendment in all conventional and organic fields every two

132 years at rates around 5000 kg/ha in vineyards, and every 2-3 years at 3500 kg/ha in olive

133 groves. All conventional and organic fields were tilled 4-6 times a year at 20-30 cm depth to

134 control weeds, and the soil was continuously bare in all systems.

135 *2.2. Soil sampling*

136 In each olive grove, five individual trees were chosen in the central area of each field. Three

137 subsamples of about 300g of soil were collected around each tree at 1.5m from the tree trunk

138 and composed into one soil sample. Thus, five composite soil samples were collected from

139 each field. In the vineyards, five vines were chosen in the central zone of each field and one

140 composed sample was taken around each plant by collecting three subsamples at 0.5m from

141 the vine rootstock. When vines were growing in metal espaliers, the subsamples were taken

142 right below the irrigation line between rootstocks.

143 The individual subsamples were taken with a shovel at 0-15 cm depth. Soil samplings occurred

144 in spring and autumn in two consecutive years (May 2013, October 2013, May 2014 and

145 October 2014). A total of 240 soil samples were collected in this study (12 fields x 5 samples x 4

146 samplings). Samples were kept at 4°C until processed. Each composite sample was divided into

147   two subsamples: 300g of fresh soil were used for nematode extraction and 500 g were air-

148   dried and used for soil physical-chemical properties.

149   *2.3. Soil properties*

150   Total soil C, N, and P, ammonium, nitrate, and bioavailable P and K concentrations, pH, and

151   electrical conductivity (EC) were measured in all samples. Total organic carbon was determined

152   by chromic acid digestion (Heanes, 1984). Total N and P were digested and extracted by the

153   Kjeldahl method (Kjeldahl, 1883). Available phosphorus was determined as Olsen P by the

154   colorimetric ascorbic acid method described by Watanabe and Olsen (1965). Bioavailable

155   potassium was extracted with ammonium acetate 1N according to Pratt (1965) and quantified

156   by an ICP spectrometer (Optima 5300 DV, Perkin Elmer). NH_4^+ and NO_3^- were extracted in KCl

157   2M. Soil pH and EC were determined in a 1:5 soil:water solution and measured in a pH-meter

158   conductimeter. Total C, N, and P, as well as NH_4^+ and NO_3^-, were then analyzed in a

159   SkalarSAN++ autoanalyzer in an accredited laboratory in the Rey Juan Carlos University

160   (Madrid, Spain).

161   *2.4. Nematode community analysis*

162   Soil nematodes were extracted from 300 g of fresh soil by sieving and Baermann funnel

163   method (Barker et al., 1985). Nematode abundance was expressed as the number of

164   individuals per 100 g of dry soil. All nematodes from each sample were counted under a

165   dissecting microscope, and at least 100 nematodes per sample were identified to genus or

166   family under the microscope. Nematodes were classified as bacterial feeders, fungal feeders,

167   plant parasites/herbivores, omnivores and predators (Yeates et al., 1993). They were also

168   classified according to the colonizer-persister (cp) scale (Bongers, 1990). The cp scale

169   comprises five groups of nematode families, namely microbial feeders with short life cycles

170   and high reproduction rates (cp 1 and cp 2) and predators and omnivores with long life cycles,

171   low reproduction rates and which are very sensitive to environmental perturbations (cp 4 and

172   5). Taxa richness (S) was expressed as the average number of taxa in each sample.

173   The Maturity Index (MI) and the Plant-Parasitic Index (PPI) (Bongers, 1990) were calculated as

174   a weighted mean of the relative contribution of each cp group to the assemblage of free-living

175   nematodes (MI) and of herbivore and plant parasitic nematodes (PPI) respectively. In addition,

176   four soil food web indices (Ferris et al., 2001) were calculated: 1) The Structure Index (SI), a

177   weighted measure of the proportion of sensitive predator and omnivore nematodes, is a

178   sensitive indicator of soil food web complexity; 2) The Channel Index (CI), based on the ratio of

179   fungivore to bacterivore nematodes, is an indicator of the prevalence of organic matter

180   decomposition mediated by fungi; 3) The Basal Index (BI), based on the abundance of general

181   opportunistic nematodes, is an indicator of basal, perturbed soil food web condition; and 4)

182   the Enrichment Index (EI), based on the abundance of enrichment opportunistic nematodes, is

183   an indicator of rapid, bacterial-mediated organic matter decomposition. The graphical

184   representation of the SI *vs* the EI allows for the diagnosis of the soil food web as disturbed,

185   maturing, structured, or degraded (Ferris et al., 2001).

186   Average nematode taxa biomass was obtained from the NINJA (Nematode Joint Indicator

187   Analysis) System (https://sieriebriennikov.shinyapps.io/ninja/) (Sieriebriennikov et al., 2014).

188   Metabolic footprints (Ferris, 2010) are based on the calculation of the lifetime amount of C

189   used by nematode taxa in growth and egg production and in C losses with respiration.

190   Metabolic footprints are indicators of the magnitude of ecosystem functions driven by

191   nematode functional guilds and trophic groups. The Enrichment, Structure, Bacterivore,

192   Fungivore, Herbivore, Predator, and Omnivore footprints were calculated using the NINJA

193   internet tool (https://sieriebriennikov.shinyapps.io/ninja/).

194   Radial diagrams, used in agroecological systems as indicator representations of agroecosystem

195   condition (Altieri, 2002; Stavi et al., 2016) were adapted to represent metabolic footprints and

196   the services/disservices associated to their magnitudes (Fig. S1; Sánchez-Moreno and Ferris, in

197   press).

198   *2.5.Data Analysis*

199   Data were log-transformed before the analyses to improve normality. The effects of sampling

200   date (May 2013, October 2013, June 2014, October 2014), type of crop (olive groves and

201   vineyards), and management system (organic and conventional) on soil properties and soil

202   food web attributes were analyzed by Factorial Analyses of Variance (ANOVA). Since

203   preliminary statistical evaluations showed that the effect of site (each site composed by one

204   organic and one conventional field, Table 1) was always not significant ($p > 0.05$) it was not

205   considered in further statistical tests. Principal Component Analysis (PCA) was performed on

206   nematode taxa abundances and soil samples were plotted on the resulting factors to detect

207   similarities among soil samples in terms of nematode community composition. Canonical

208   Analysis of Correspondence (CCA) was used to infer relationships between nematode taxa

209   abundance and categorical (crop type, management) and continuous (total N, C, and P,

210   available P and K, electrical conductivity, NH_4^+, NO_3^-, soil moisture, pH) independent variables.

211   Only nematode taxa present in more than one sample were subject to multivariate analyses.

212   Discriminant Analysis was used to ascertain the discrimination ability of soil properties and

213   food web attributes; the resulting discriminant models were assessed through the p-value

214   associated to the Wilk's Lambda, which ranges from 0 (perfect discrimination) to 1 (no

215   discrimination at all). Resulting Squared Mahalanobis Distances among groups were used to

216   construct a cluster tree to visualize similarities among field types. All statistical analyses were

217   performed with the Dell Statistica software package Inc (2016).

218

219   **3. Results**

220

3.1. Soil properties

Results of the factorial ANOVA showed that sampling date and crop type (olive vs vineyard)

significantly affected all soil properties, while the type of system (conventional or organic)

affected all properties except NH_4^+ and soil moisture ($p<0.05$, Table S1). The bi-factorial

interactions with sampling date (date x crop, date x system) significantly affected half of the

properties, while crop x system and the interaction of all variables (date x crop x system) have

minor or no effects on soil properties (Table S1). In general, soil properties tended to present

higher values in vineyards than in olive groves and in conventional than in organic fields (Fig.

1).

3.2. Microfaunal food webs

Forty-three nematode taxa (16 bacterivores, 7 fungivores, 11 herbivores, 3 omnivores, 5

predators, and 1 entomopathogenic nematode) were identified in the study fields (Table 2).

Per trophic group, the most abundant nematode taxa were *Acrobeloides* (bacterivore),

Aphelenchus, *Aphelenchoides*, and Tylenchidae (fungivores), *Pratylenchus* and *Paratylenchus*

(herbivores), Qudsianematidae (omnivores) and *Discolaimus* (predators), all being in general

more abundant in olive groves than in vineyards (Table 2). Nematode abundance varied across

sampling dates, type of crop and system (Table 2). Only the date x crop interaction significantly

affected a large number of taxa (Table 2).

Composition of the nematode community differed between olive groves and vineyards and

between organic and conventional vineyards according to the PCA, with smaller differences

between organic and conventional olive groves (Fig. 2). The first two PCA axis explained 11.0%

and 6.2% of composition variation, respectively.

Soil properties affecting nematode abundance that led to differences among the four systems

are summarized in the CCA (Fig. 3). In general, crop type affected nematode abundances to a

245 larger extent than management system did. *Tylencholaimus*, Qudsianematidae, *Cervidellus*,

246 *Paratylenchus*, and *Helicotylenchus* were associated to higher NH_4^+ and NO_3^- concentration in

247 organic fields. Total N and P, soil moisture, and Olsen P were associated with conventional

248 vineyards and inversely associated with *Achromadora*, *Tylenchorhynchus*, and *Pratylenchus*.

249 *Meloidogyne* was associated to vineyards and *Acrobeloides*, *Aphelenchus*, and Tylenchidae to

250 olive groves (Fig. 3).

251 Nematode density and taxa richness were in general low, with a mean nematode density of

252 342.3 nematodes/100g dry soil, and an average of 10.3 taxa per sample. Nematode abundance

253 was on average two to three-fold higher in olive groves than in vineyards, regardless of the

254 type of management (Table 3). Sampling date showed a significant effect on all soil food web

255 descriptors, while there were several differences between crop types and few between

256 management systems. The Enrichment Index, indicator of the prevalence of enrichment-

257 opportunistic species, and the Plant-parasitic Index, indicator of nematode pest pressure, were

258 higher in vineyards than in olive groves (Table 3). Taxa richness, nematode abundance, and the

259 Basal Index, indicator of perturbed soil food web condition, were higher in olive groves than in

260 vineyards (Table 3). Only two descriptors varied significantly between systems, with higher

261 values of the Maturity Index and the Plant-parasitic Index in organic than in conventional fields

262 (Table 3). The interaction sampling date x crop type and crop x system affected some soil food

263 web measures, in contrast to the interaction sampling date x management system, which did

264 not affect the soil food web (Table 3).

265 *3.3. Diagnosis of soil food web function*

266 The graphical diagnosis of soil food web condition deemed the four systems as degraded. The

267 metabolic footprints varied among crop types and management systems, and vineyards

268 presented a reduced functionality compared to olive groves (Fig. S2).

269   Relevant seasonal variations were found. In May 2013 (Fig. 4) herbivore metabolic footprint

270   was higher in organic than in conventional systems, influenced by crop (ANOVA F-value =

271   4.57), management (ANOVA F-value = 4.33), and their interaction (ANOVA F-value = 3.56) (all

272   p-values <0.05). All systems presented a low predator footprint, affected by crop type (ANOVA

273   F-value = 4.50, p<0.05) and crop x system interaction (ANOVA F-value = 4.59, p<0.05).

274   Bacterivores were more active in conventional than in organic systems (ANOVA F-value = 4.23,

275   p<0.05).

276   In October 2013 (Fig. 4) the footprint of enrichment indicators (ANOVA F-value = 11.43),

277   fungivores (ANOVA F-value = 16.38), and bacterivores (ANOVA F-value = 17.90) (all p-values

278   <0.05) were higher in olive groves than in vineyards. The next spring herbivore pressure was

279   large again, and footprints of bacterivores (ANOVA F-value = 18.78), fungivores (ANOVA F-

280   value = 42.27), omnivores (ANOVA F-value = 6.59), and structure indicators (ANOVA F-value =

281   6.89) (all p-values <0.05) were higher in olive groves than in vineyards, while enrichment

282   indicators responded to crop type (ANOVA F-value = 15.57, p<0.05) and crop x system

283   interaction (ANOVA F-value = 7.21, p<0.05). At the last sampling date bacterivores (ANOVA F-

284   value = 74.38), fungivores (ANOVA F-value = 33.10), herbivores (ANOVA F-value = 9.72), and

285   structure indicators (ANOVA F-value = 42.4) (developed larger activity in olive groves than in

286   vineyards, while fungivores were too more active in organic than in conventional systems

287   (ANOVA F-value = 5.12) (all p-values <0.05).

288   Soil properties presented large discrimination capability (Wilks' Lambda = 0.12, p<0.05), with

289   all soil properties except total C contributing (p<0.05) to the discrimination power of the

290   model (Fig. S3). Nematode taxa also discriminated well among groups (Wilks' Lambda =0.19,

291   p<0.05), separating olive groves and vineyards in different clusters, but soil food web indices

292   discriminated poorly among field types (Wilks' Lambda =0.86, p<0.05), grouping fields

293   idiosyncratically (Fig. S3). Metabolic footprints presented higher discrimination ability (Wilks'

294   Lambda = 0.58, p<0.05), and grouping vineyards and olive groves in different clusters (Fig. S3).

295   All discriminant models showed larger differences between olive groves and vineyards than

296   between conventional and organic fields.

297

298   **4. Discussion**

299

300   *4.1. Relationship between management type and soil properties*

301   Purported benefits of organic agriculture compared to other management practices include

302   increased soil C and N concentration, soil fertility, water retention, and overall provision of

303   ecosystem services (Garbach et al., 2017). In contrast to such findings, conventional

304   management increased soil C, N, P and K stocks in the studied fields, and vineyards presented

305   higher quantities of nutrients than olive groves. Higher nutrient inputs in the form of mineral

306   fertilizers in conventional fields may explain the observed differences for N, P and K. However,

307   since both conventional and organic fields received similar amounts of organic amendments

308   (manure), detected differences in soil C cannot be explained with our data. In the studied

309   fields, organic management did not improve soil nutrient condition. Soils in Mediterranean

310   woody crops commonly present low organic matter levels (Vicente-Vicente et al., 2016), with

311   typical C contents below 1% in different olive-growing areas in Spain (Álvarez et al., 2007;

312   Benitez et al., 2006; Gómez et al., 1999; Gómez et al., 2009). In contrast to other studies,

313   differences between organic and conventional fields in our study related exclusively to the

314   absence of synthetic pesticides or fertilizers in the fields, but organic management was not

315   accompanied by any other practice to increase soil protection or soil diversity such as cover

316   cropping or reducing tillage; thus, the absence of chemical pesticides and fertilizers was not

317   enough to induce a positive response in soil fertility. In agreement with our results, several

318   previous studies have shown small differences in soil properties between organic and

319   conventional systems, as found in a long-term experiment in Switzerland (Mäder et al., 2002),

320   and in a multiple comparisong among farms in The Netherlands (van Diepeningen et al., 2006).

321   *4.2. The nematode community response to crop type and soil management*

322   Temporal dynamics of the nematode community was obvious during our study. In horticultural

323   systems, nematode population dynamics are commonly determined by the crop cycle, crop

324   species, and root condition (Scharroba et al., 2016). In our perennial systems such effects are

325   probably weaker but still noticeable. Besides, nematode taxa varied more between crops than

326   between management systems, which barely affected the microfaunal soil food web in our

327   study. Previous studies, however, have found shifts in nematode community composition

328   induced by the farming system that exceeded crop-related assemblage shifts (Quist et al.,

329   2016). In our study, nematode taxa driving differences between olives and vineyards, and, to a

330   smaller extent, between organic and conventional vineyards, were the most common

331   nematodes, belonging to different trophic groups, and not to the presence or absence of the

332   scarce ones, hinting the existence of similar ecological niches in different systems and similar

333   resource availability between systems.

334   Mineral and organic fertilizers commonly result in clear effects on soil fauna (Zhang et al.,

335   2016b), whereas mineral fertilizers may reduce bacterivore nematodes (Bulluck III, 2002) due

336   to direct toxicity of nitrogenous solutions (Tenuta and Ferris, 2004). Higher abundance of

337   microbial-feeding nematodes in olive groves than in vineyards in this study, which cannot be

338   attributed to soil amendments, might relate to plant-specific attributes such as root exudates,

339   litter input, and crop-specific management. Root exudates are known to affect soil biota (van

340   Dam and Bouwmeester, 2016) and to determine, for example, plant-parasitic nematode

341   infection abilities (Yang et al., 2016). The extent to which such species-specific attributes affect

342   soil biota under different fertilization regimes is unexplored.

343 Microbial feeding nematode taxa showed no clear response to management. Plant-feeding

344 nematodes, on the contrary, were more abundant in organic than in conventional plots. In

345 previous studies, we found higher abundance of different bacterivore taxa in organic than in

346 conventional plots in horticultural systems, in which at least two plant-pathogens were also

347 enhanced by organic management (Sánchez-Moreno et al., 2009). In contrast, many studies

348 report a better regulation of plant-parasitic nematodes in organic than in conventional systems

349 (Briar et al., 2007), as well as higher contribution of bacterivore in detriment of herbivore

350 nematodes in organic than in conventional plots (Tsiafouli et al., 2007; Benković-Lačić et al.,

351 2016), but such positive effects of organic management in microfaunal soil food webs were not

352 found in our study. Indeed, although previous studies in horticultural crops reported that

353 different nematode pathogens may demonstrate contrasting response to organic management

354 (Clark et al., 1998). Root-hair feeders (Tylenchidae), ectoparasites (*Paratylenchus*,

355 *Tylenchorhynchus* and *Xiphinema*), and endoparasites (*Pratylenchus*) showed higher

356 abundance in organic compared to conventional soils in our study. Consistent patterns,

357 however, cannot be found in the literature, and while in northern latitudes nematodes seem

358 to respond positively to organic farming (Mulder et al., 2003), in Mediterranean sites with low

359 precipitation and soil organic carbon, free-living nematodes did not respond to organic

360 management, and plant-feeding nematodes might increase in organic fields (Coll et al., 2011).

361 Also in accordance with our results, some parasitic nematodes as *Meloidogyne* and

362 *Helicotylenchus* were found to be more abundant in organic than in conventional tree crops in

363 semiarid regions of USA (Pokharel et al., 2015).

364 *4.3. Impact on soil functioning*

365 Our results show that soil food web indices and nematode metabolic footprints, although

366 based on similar theoretical frameworks (Ferris, 2010; Ferris et al., 2001), offer complementary

367 information. Soil food web indices reflect structural attributes of the nematode community,

368   while metabolic reflect the magnitude of the ecological functions performed by nematodes

369   participating in different ecological functions. In our study, the Structure Index indicated an

370   overall degraded situation in all fields. Varying nematode biomass across fields, however, led

371   to different structural metabolic footprints across systems, hinting different ecosystem

372   functioning. The structure metabolic footprint, an indicator of soil suppressiveness (Steel and

373   Ferris, 2016) was larger in olive groves than in vineyards. To increase soil suppressiveness,

374   reducing soil disturbance is mandatory to allow predatory nematodes to survive, while positive

375   nutrient-mediated bottom-up effects would be required to increase resources available to

376   higher trophic links to increase their biomass (Ferris *et al.*, 2012a).

377   Similarly, the Enrichment Index was lower in conventional olive groves than in the other three

378   systems. The higher metabolic footprints of bacterivores, fungivores, and enrichment

379   indicators in olive groves indicated higher soil fertility and a larger contribution of nematodes

380   to nutrient mineralization (Gebremikael et al., 2016) than in vineyards. Higher mineral nitrogen

381   soil content might be linked to higher biomass of bacterial-feeding, cp-1 nematodes through

382   increased microbial biomass, and higher nematode biomass might increase soil ammonium

383   through excretion (Standing et al., 2006; Briar et al., 2007; Sánchez-Moreno et al., 2008) in

384   organic olive groves. In microcosms, nematodes have been found to increase total mineral

385   nitrogen up to 32% (Gebremikael et al., 2014). Incorporation of manure or straw into the soil

386   may induce an increase on nematode biomass compared to mineral fertilizers (Zhang et al.,

387   2016b), as might have happened in organic systems, which presented larger enrichment

388   footprints in three out of the four sampling dates.

389   Herbivore pressure was higher in vineyards than in olive groves, and herbivore activity was

390   higher in the spring than in the fall, probably mediated by climatic conditions and the plant

391   phenological stage. Herbivore pressure, however, cannot be directly related to plant damage,

392   since the outcome of herbivory will result from a complex interaction between herbivores,

393 natural enemies, and plant condition, and will be greatly affected by soil food web structure

394 (Macfadyen et al., 2009).

395 *4.4. Does organic management improve soil health?*

396 Vineyards and olive groves are often established in areas of low productivity and vulnerable

397 soils. In the fields included in this study, organic management did not result in obvious

398 improvement of soil quality. Bare soils, continuous tillage operations and few organic

399 amendments are probably responsible of such lack of improvement (Calabrese et al., 2015;

400 Fernández-Romero et al., 2016; Laudicina et al., 2016). In a previous study in Southern Spain,

401 we found that tillage, herbicides, and bare soils depleted soil food webs in olive groves, and

402 these negative effects affected nematodes in higher trophic links (Sánchez-Moreno et al.,

403 2015). In this study, none of the indicators used clearly discriminated between organic and

404 conventional systems, and crop-related differences overcome the slight differences found

405 between management systems.

406 Other management techniques such as weed mowing, cover cropping, and reduced tillage,

407 have been shown to improve significantly soil quality, soil diversity, microbial populations, soil

408 C concentration, and beneficial nematodes in semi-arid conditions (Gómez et al., 2009;

409 Henneron et al., 2014; Simoes et al., 2014; Moreno and Benitez, 2016). In Southern Europe,

410 no-till and reduced-till are well established techniques among organic farmers, but green

411 manures are seldom used (Peigné et al., 2016). Seufert et al. (2017) have recently highlighted

412 that narrow focus of organic regulations worldwide do not incorporate the original concept of

413 organic farming as a holistic farming system to improve soil health.

414 Studying the effects of organic management in a wider variety of woody crops and in larger

415 study area would be necessary to infer to what extent organic farming enhances soil and

416 ecosystem conditions under semi-arid conditions. A holistic view of organic farming, including

417 the use of carbon-based amendments, diverse crop rotations, and cover cropping (Reeve et al.,

418   2016) might be required to benefit from soil and ecosystem health in the form of food.

419   Preserving and improving agroecosystem health should be considered a priority goal in

420   landscape management in Southern Europe, since olive orchards and vineyards play a crucial

421   role in the maintenance of traditional agricultural landscapes together with local identity,

422   history and economy (Biasi et al., 2012).

423   **5.   Conclusions**

424   The few differences found between the organic and conventional vineyards and olive orchards

425   included in our study suggest no obvious benefit from organic farming in terms of soil

426   properties, particularly soil fertility, and soil food web structure. The effects of crop species

427   was much noticeable than the effects of system management. Of the indicators used, none

428   was able to discriminate clearly between conventional and organic systems. Differences

429   among sampling dates indicated strong temporal patterns of nematode community dynamics

430   in these semiarid perennial crops. In the semi-arid conditions of this study, active soil

431   protection such as that provided by no-tillage or cover crops are probably needed to obtain

432   the environmental benefits pursued by organic farming.

440

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Figure 1.
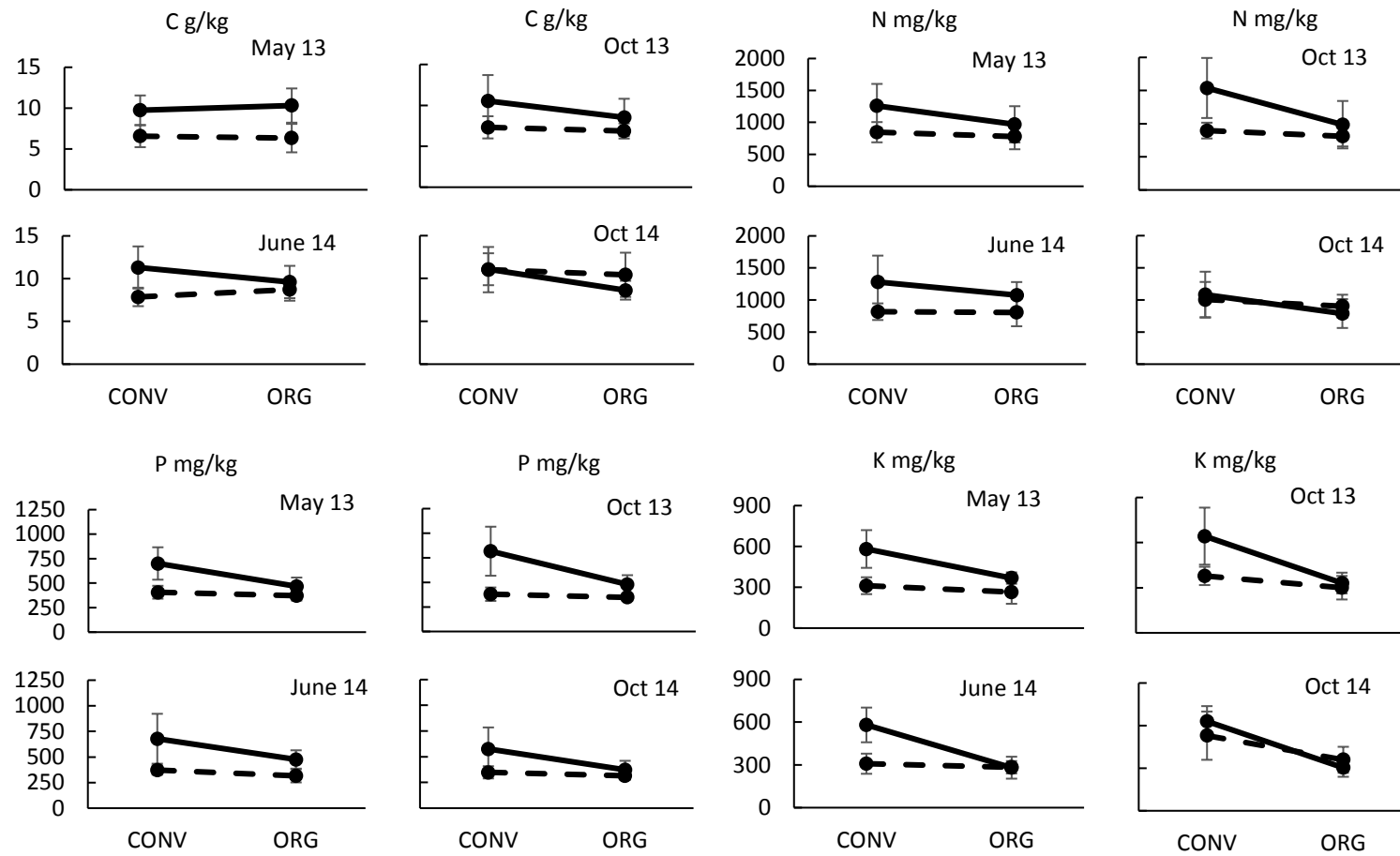
Figure 1. Continuation
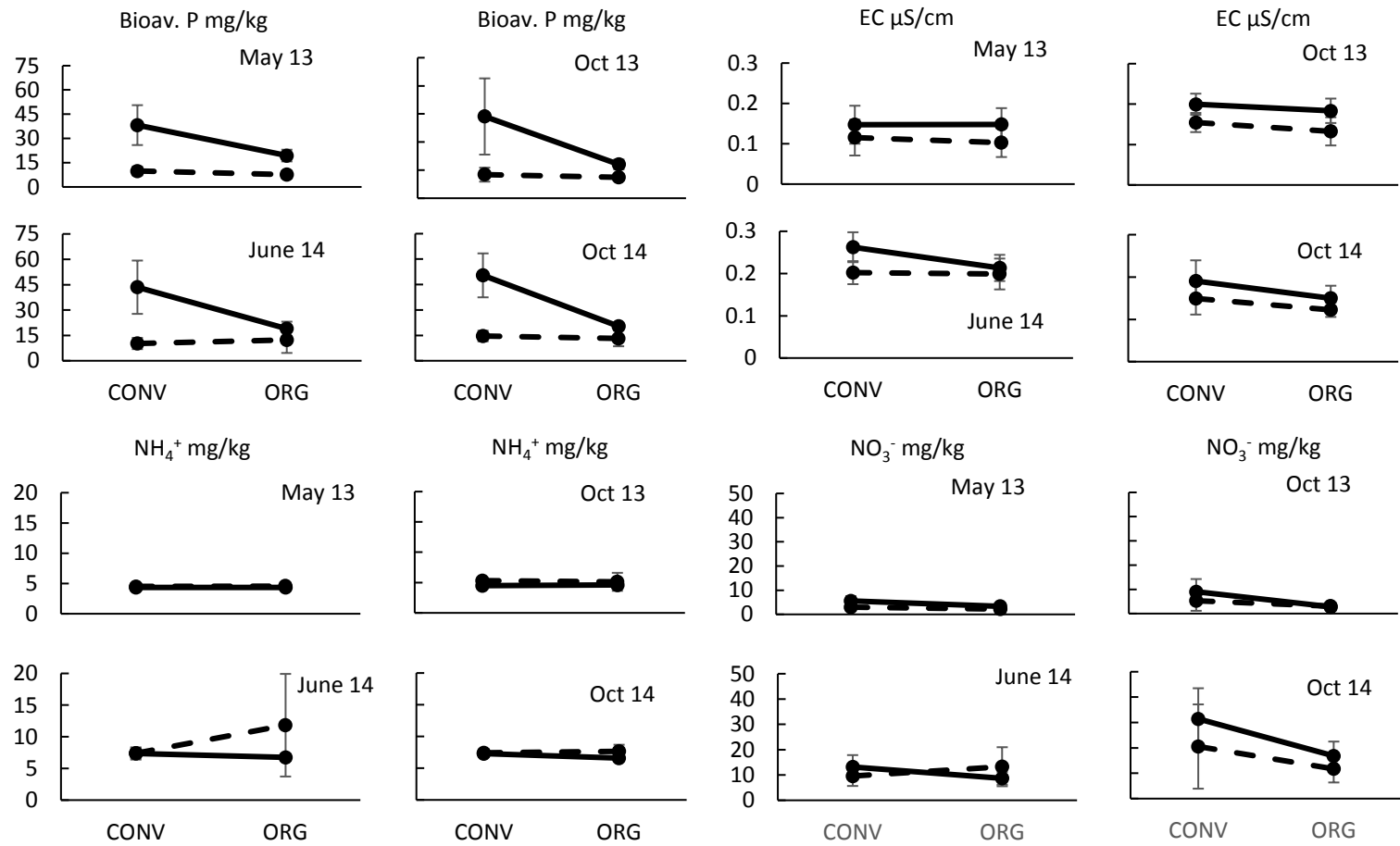
Figure 1. Continuation
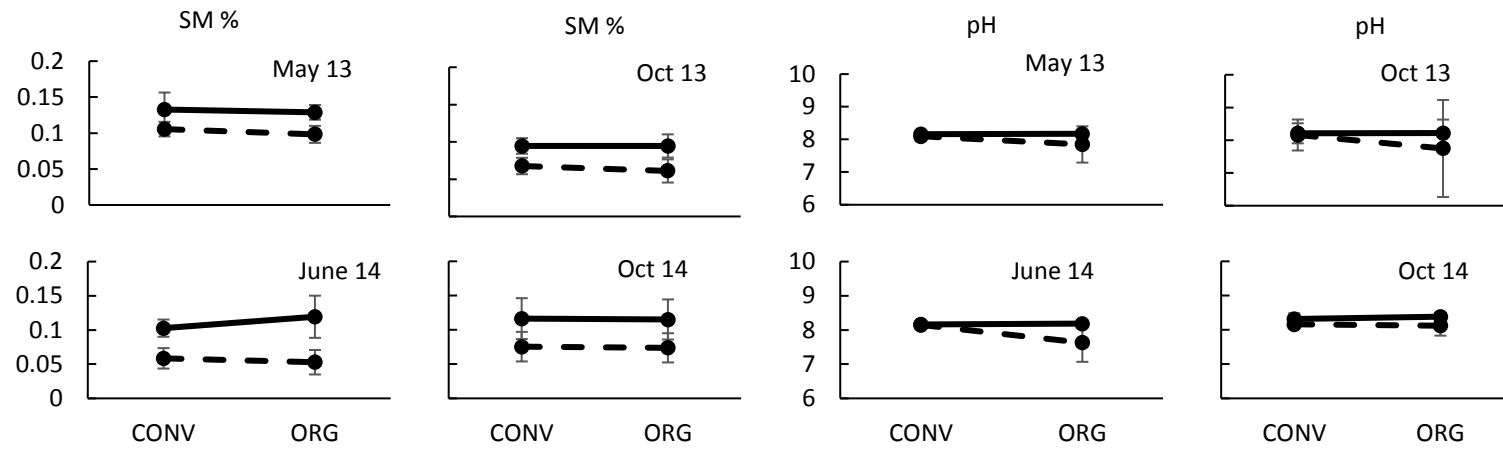
Figure 2

Figure 3.

Figure 4.

Legend: − ■ − CONV. OLI.   − ● − ORG. OLI.   ─■─ CONV. VINE.   ─●─ ORG. VINE.

May 2013 — Herb. Footp., Omn. Footp., Struct. Footp., Pred. Footp., Fung. Footp., Enrich. Footp., Bacteriv. Footp.

Oct. 2013 — Herb. Footp., Omn. Footp., Struct. Footp., Pred. Footp., Fung. Footp., Enrich. Footp., Bacteriv. Footp.

June 2014 — Herb. Footp., Omn. Footp., Struct. Footp., Pred. Footp., Fung. Footp., Enrich. Footp., Bacteriv. Footp.

Oct. 2014 — Herb. Footp., Omn. Footp., Struct. Footp., Pred. Footp., Fung. Footp., Enrich. Footp., Bacteriv. Footp.
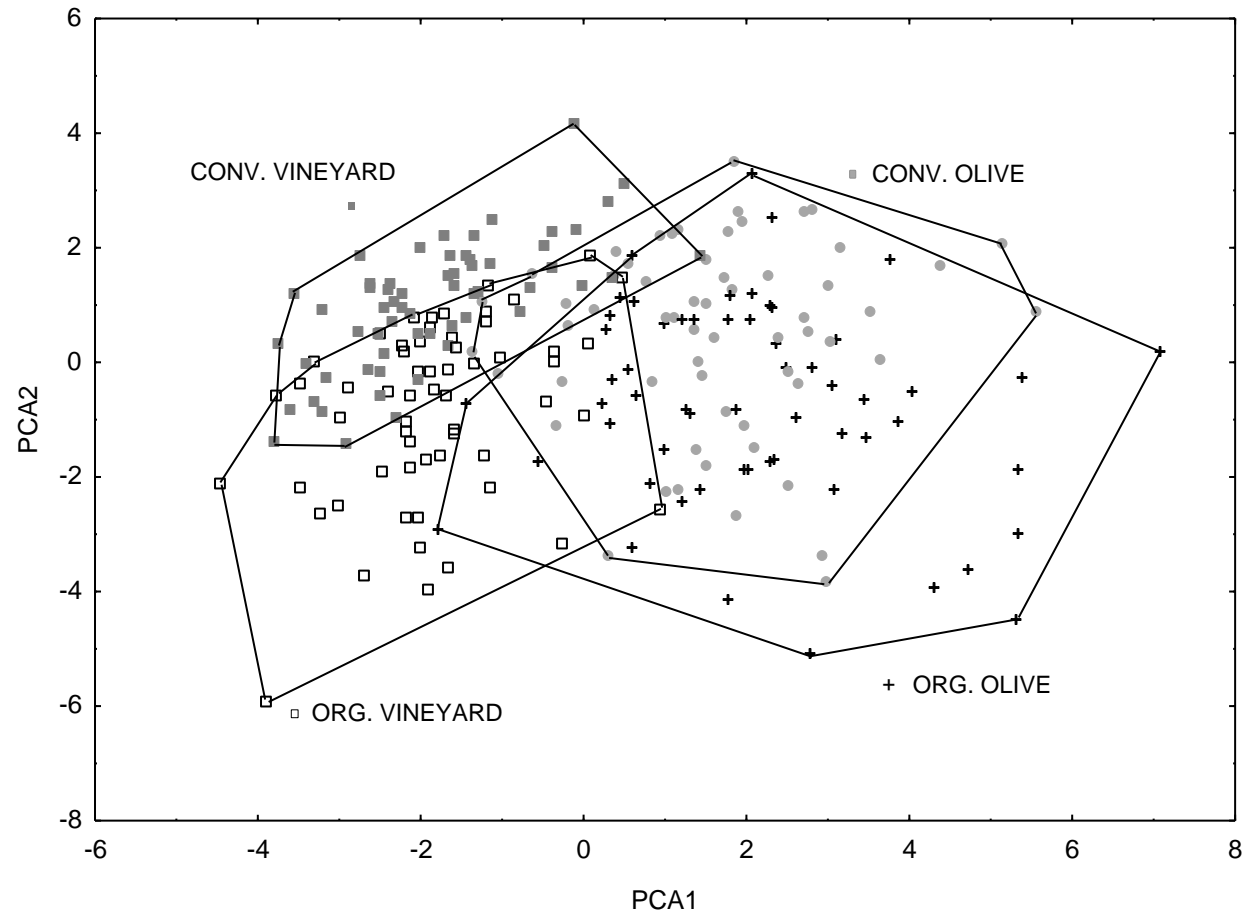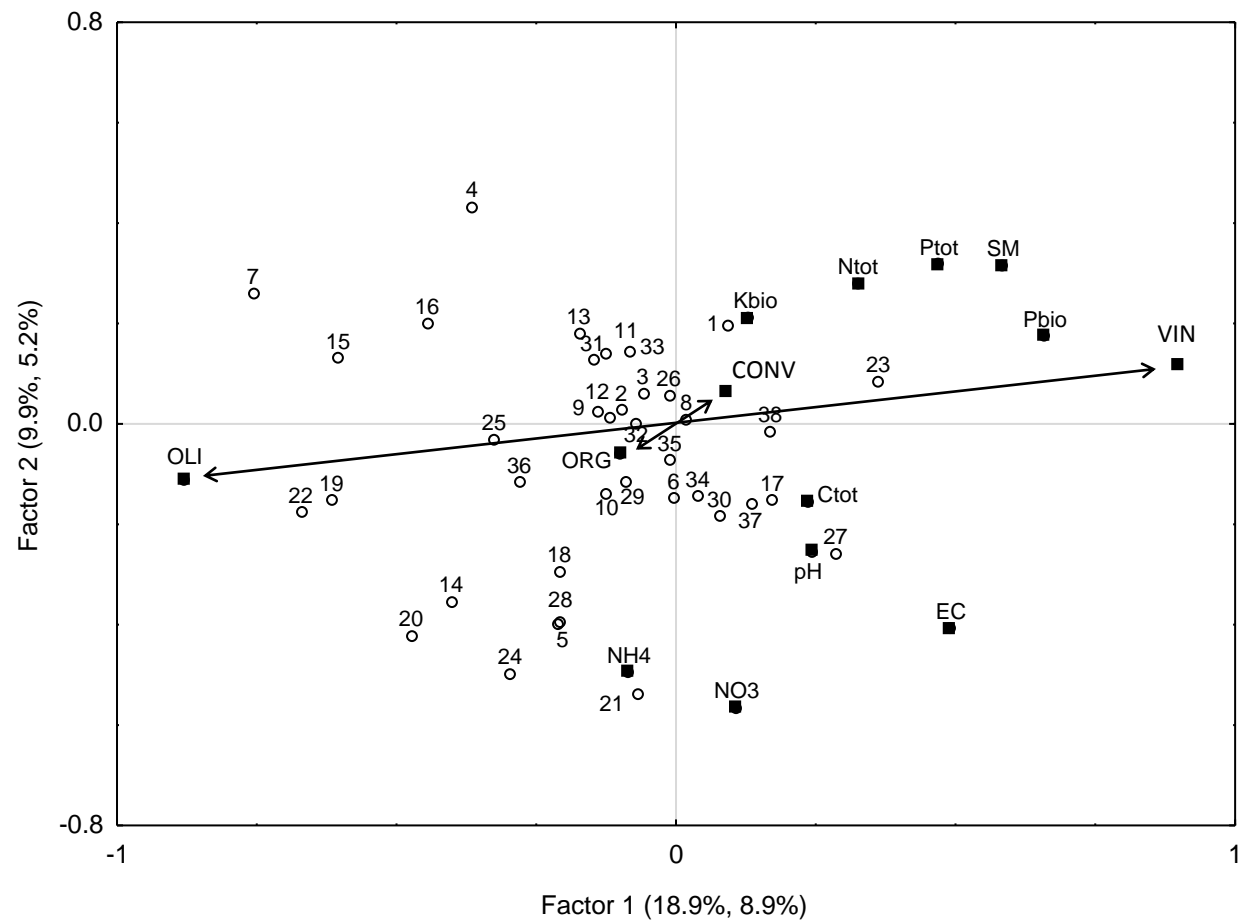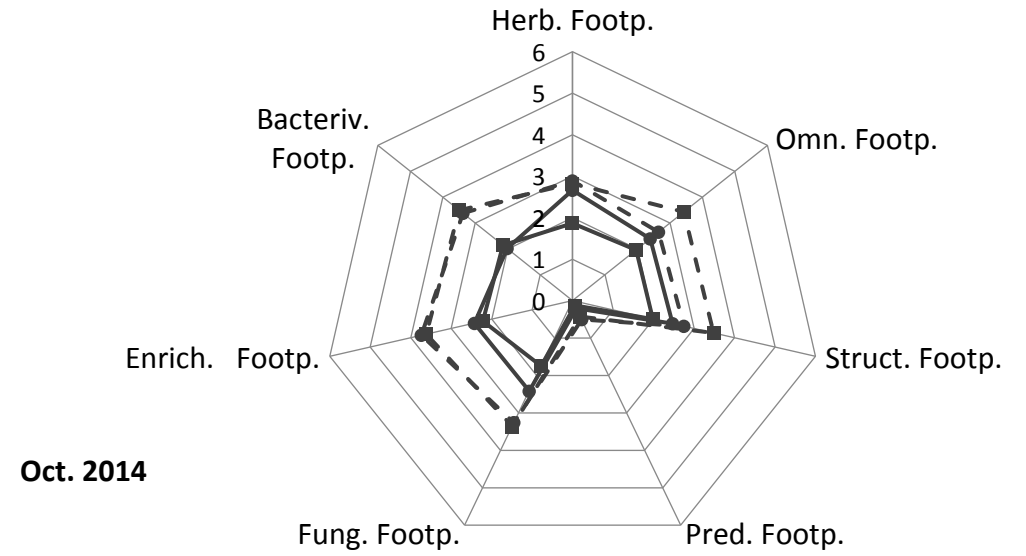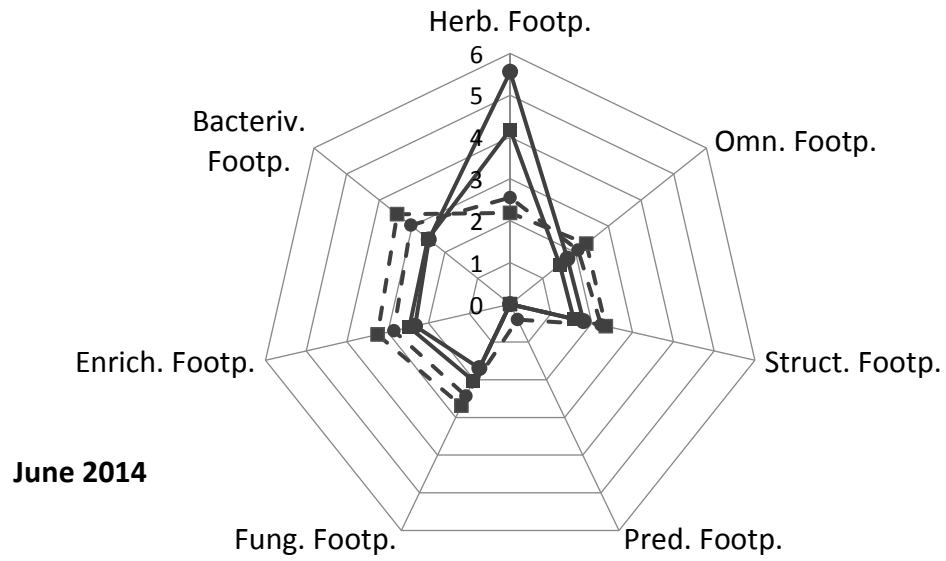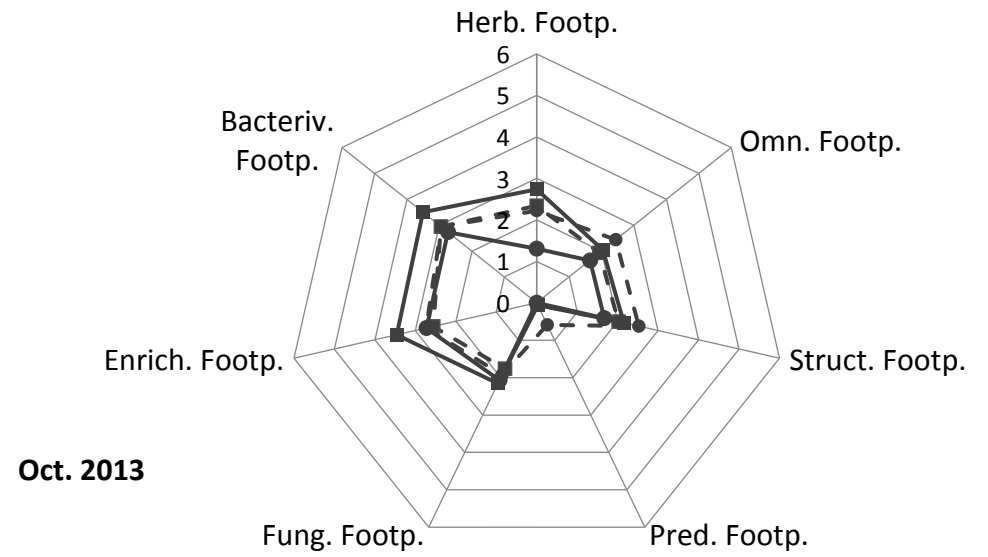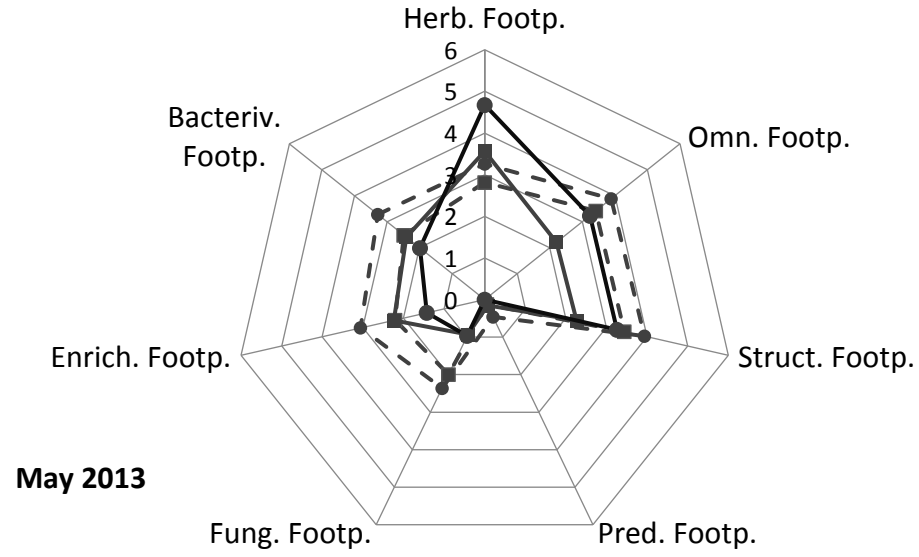
Figure legends

Fig. 1. Mean values (±SE) of soil C (g/kg), soil N (mg/kg), soil P (mg/Kg), soil bioavailable K (mg/kg), bioavailable P (mg/kg), electrical conductivity (EC, µS/cm), NH_4^+ (mg/kg), NO_3^- (mg/kg), soil moisture (SM, %), and soil pH at four sampling dates (May 2013, October 2013, June 2014, October 2014) in conventional (CON) and organic (ORG) vineyards (solid line) and olive groves (broken line) . NH_4^+ and NO_3^- are differently scaled in 2013 and 2014 data to facilitate visualization. For statistical comparisons, see Supplementary material (Table S1).

Fig. 2. Ordination of soil samples on the biplot resulting from the Principal Component Analyses based on the nematode community composition of soil samples. Samples of the four systems are delimited (CONV = Conventional, ORG = Organic) to facilitate interpretation.

Fig. 3. Results of the Canonical Correspondence Analysis showing the associations between soil properties (Ctot = total C, Ntot = total N, Ptpt = total P, Pbio= bioavailable P, K = bioavailable K, EC = electrical conductivity, SM = soil moisture, NO3= NO_3^-, NH4= NH_4^+), crop type (VIN = vineyards, OLI = olive groves), and management system (ORG = organic, CONV = conventional), and nematode taxa (1: Dauerlarvae, 2: *Mesorhabditis*, 3: *Rhabditis*, 4: *Panagrolaimus*, 5: *Cervidellus*, 6: *Acrobeles*, 7: *Acrobeloides*, 8: *Metateratocephalus*, 9: *Plectus*, 10: *Wilsonema*, 11: *Eumonhystera*, 12: *Chiloplacus*, 13: *Prismatolaimus*, 14: *Achromadora*, 15: *Aphelenchus*, 16: *Aphelenchoides*, 17: *Diphterophora*, 18: *Tylencholaimus*, 19: Tylenchidae, 20: *Tylenchorhynchus*, 21: *Paratylenchus*, 22: *Pratylenchus*, 23: *Meloidogyne*, 24: *Helicotylenchus*, 25: *Rotylenchus*, 26: *Trichodorus*, 27: *Xiphinema*, 28: Qudsianematidae, 29: *Mesodorylaimus*, 30: Aporcelaimidae, 31: *Tripyla*, 32: *Mylonchulus*, 33: *Clarkus*, 34: *Axonchium*, 35: *Discolaimus*, 36: *Aprutides*, 37: *Alaimus*, 38: *Steinernema*). Only nematode taxa appearing in more than one sample were included in the analysis. Percentage of variance explained of independent (soil properties, crop type, and management system) and dependent (nematode taxa) variables is indicated for each axis.

Fig. 4. Representation of the Herbivore, Omnivore, Structure, Predator, Fungivore, Enrichment, and Bacterivore footprints (ln-transformed, mean values) inferred in the four systems (CONV = Conventional, ORG = Organic, VINE= Vineyards, OLI = Olive groves) at four different sampling dates.

Table S1. Results of the factorial ANOVA (F statistic and p value) showing the significance of the effect of date of sampling (May 2013, October 2013, May 2014 and October 2014), crop (olive groves and vineyards), system management (organic and conventional) and their interactions on soil properties (Bioav. P and K = bioavailable P and K, EC= electrical conductivity, SM= soil moisture).

		Date (D)	Crop (C)	System (S)	D x C	C x S	D x S	D x C x S
Total C	F	16.01	53.80	7.45	14.19	1.90	5.70	2.02
(mg/kg)	p	***	***	***	***	ns	**	ns
Total N	F	3.76	14.24	6.45	0.77	0.31	0.19	0.86
(mg/kg)	p	**	***	**	ns	ns	ns	ns
Total P	F	6.75	38.25	14.19	1.98	0.32	1.35	0.69
(mg/kg)	p	***	***	***	ns	ns	ns	ns
Bioav. P	F	11.63	610.44	129.97	3.19	0.62	83.97	1.90
(mg/kg)	p	***	***	***	**	ns	***	ns
Bioav. K	F	10.73	82.44	197.59	10.14	2.59	40.06	2.16
(mg/kg)	p	***	***	***	***	ns	***	ns
NO_3^-	F	142.44	25.54	39.64	3.88	4.66	13.45	0.82
(mg/kg)	p	***	***	***	***	***	***	ns
NH_4^+	F	104.65	15.53	0.21	2.01	2.20	3.75	4.79
(mg/kg)	p	***	***	ns	ns	ns	ns	ns
pH	F	5.31	39.75	14.30	0.29	0.21	20.64	1.27
	p	***	***	***	ns	ns	***	ns
EC	F	70.74	73.28	21.44	0.51	1.69	1.05	1.95
µS/cm	p	***	***	***	ns	ns	ns	ns
SM	F	44.59	242.68	0.40	6.36	0.86	2.71	0.92
(%)	p	***	***	ns	***	ns	ns	ns

Fig. S1. Representation in a radial chart of nematode metabolic footprints (Herbivore, Omnivore, Structure, Predator, Fungivore, Enrichment, and Bacterivore footprints). The representation of the ln-transformed footprints results in a polygon in which the upper part represents the pest pressure disservice whereas the right and left parts represent the pest suppression and mineralization services, respectively. Thus, the radial representation of nematode metabolic footprints visualizes the differences among functional magnitudes of different food web links and the services/disservices in which they are involved. An optimal food web, with large magnitudes in the pest control and mineralization services and a low pest pressure disservice would present a radial diagram with a cup shape. Two hypothetical food webs are represented: the solid line represents a food web in which the fast, bacterial-mediated mineralization function and the pest pressure is high, and the pest suppression service is weak. The broken line represents a food web in which both the mineralization and the pest suppression services are strong and the herbivore pressure is low.
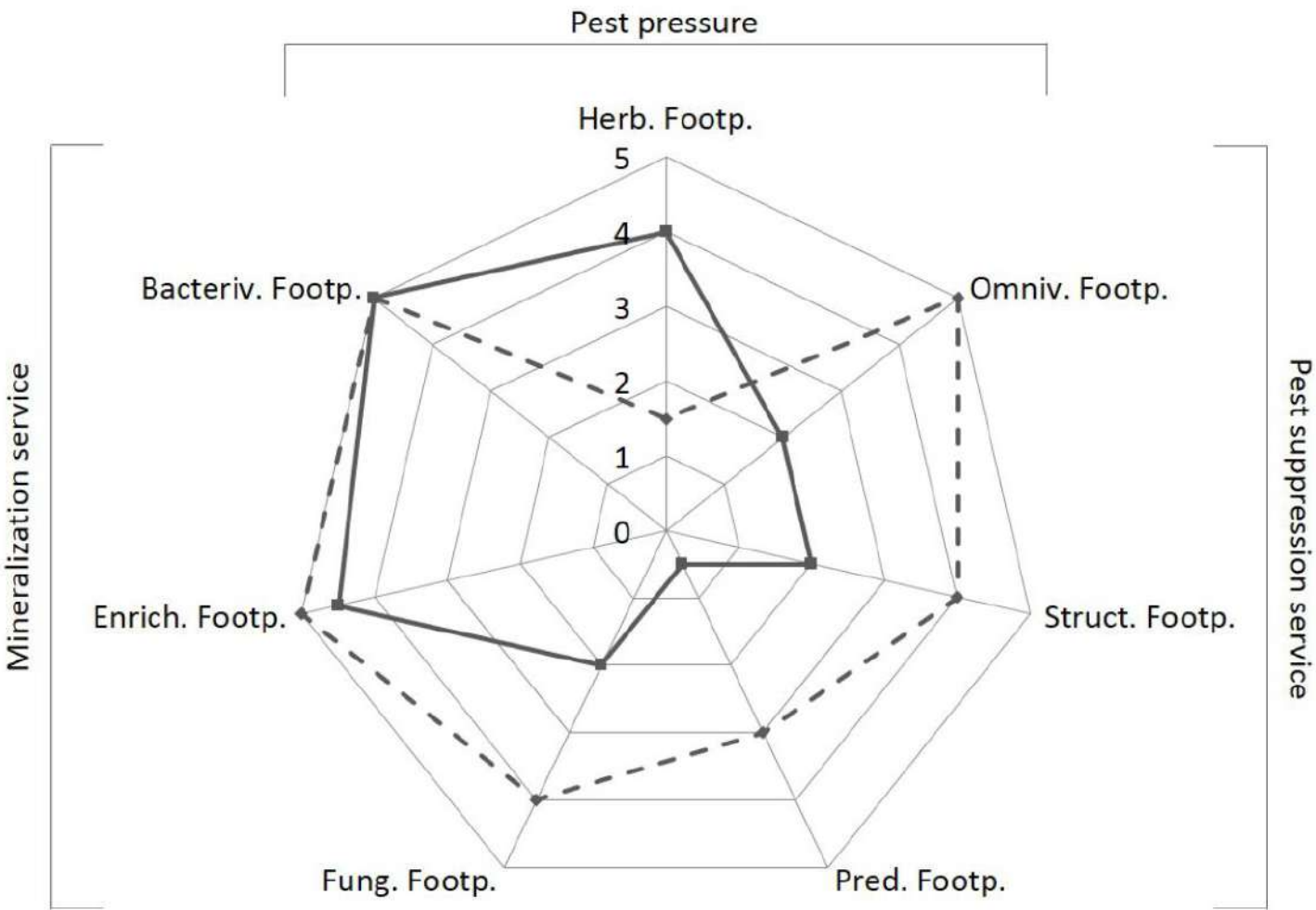
Fig. S2. Soil food web diagnosis and Structure and Enrichment Metabolic Footprints of the four crop and management systems. SI and EI values are indicated by the point in the center of each polygon. Height and width of each polygon represents the magnitude of the Enrichment and Structure Metabolic footprints.
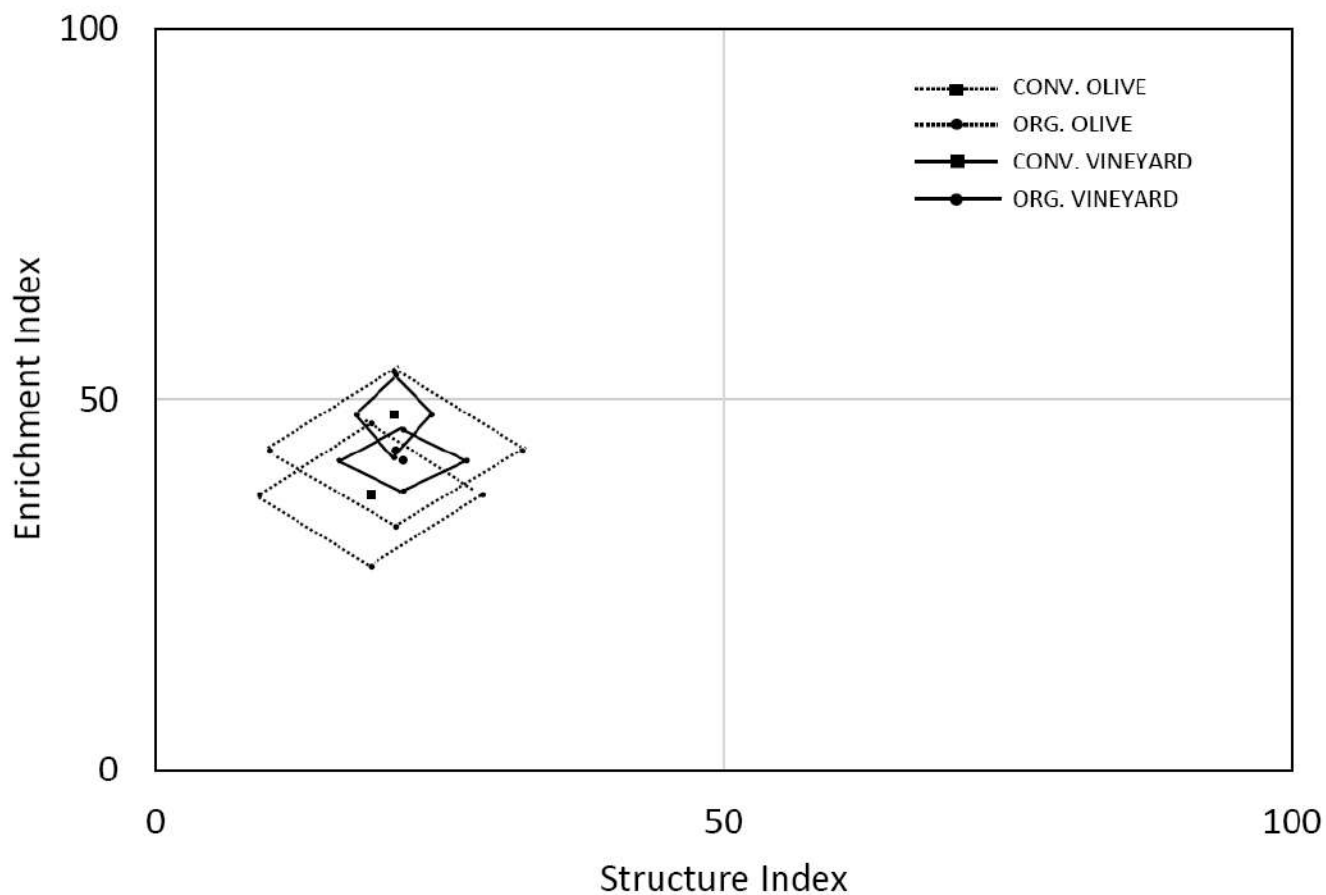
Fig. S3. Cluster analysis of the Mahalanobis distances between groups obtained by the Discriminant analysis performed on soil properties, nematode taxa, soil food web indices, and metabolic footprints. Different letters below each system type indicates significant differences between groups detected in the Discriminant analysis.
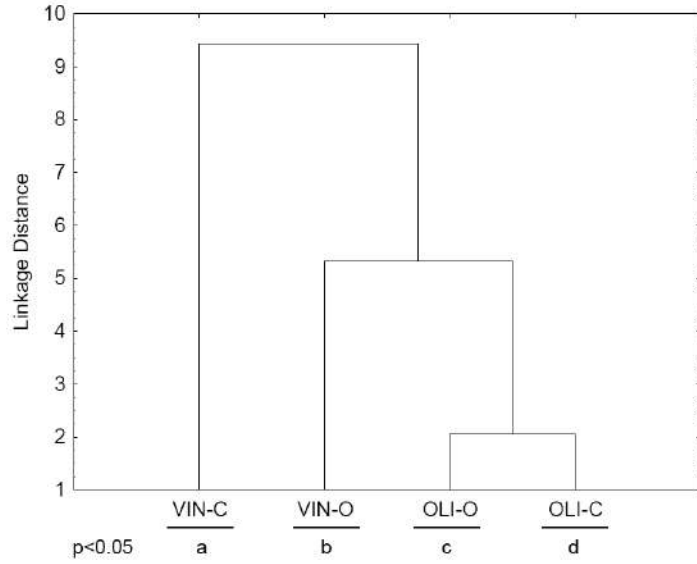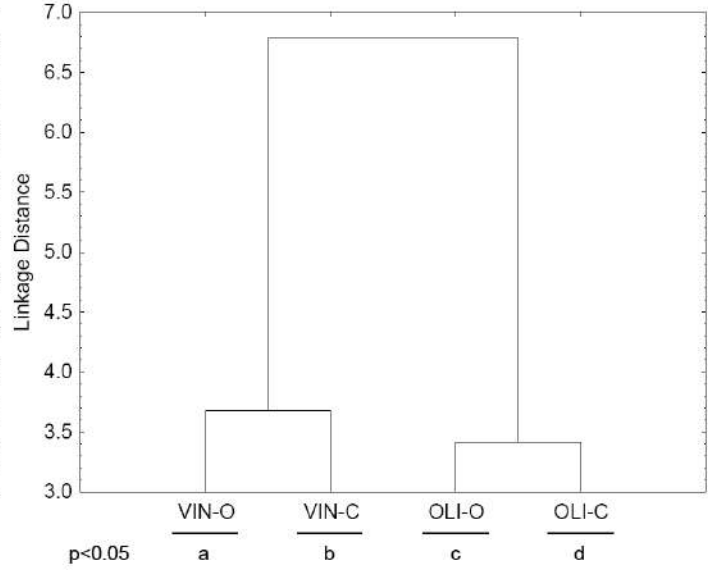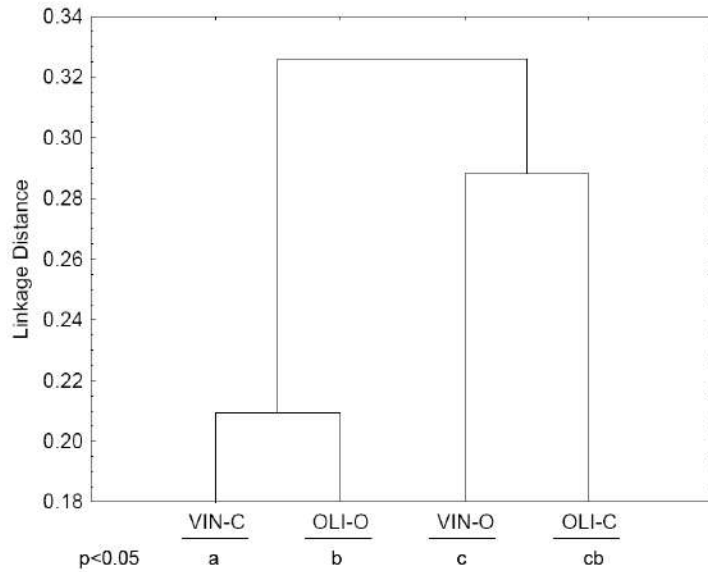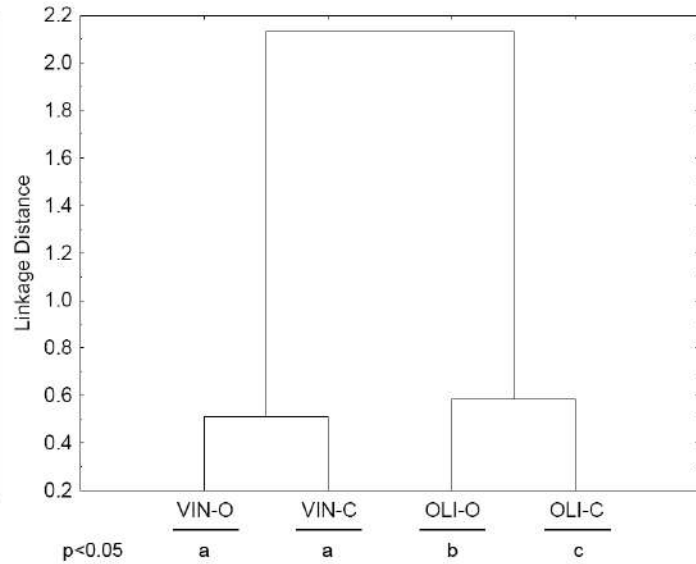
Table 1. Location, type of management, field size, and type of irrigation of the studied farms. No. of trees per hectare (in olive groves) and training system (in vineyards) is indicated.

Olive	Location	Management	ha	No. trees/ha	Irrigation
Site 1	38° 49' 19'' N	ORG	2.8	50	rainfed
	3° 16' 41'' W	CONV	1.4	50	rainfed
Site 2	38° 49' 19'' N	ORG	1.6	70	rainfed
	3° 16' 34'' W	CONV	1.6	80	rainfed
Site 3	38° 48' 48''	ORG	2.7	100	rainfed
	3° 12' 42''	CONV	3.0	100	rainfed
Vineyard	Location	Management	ha	Training	Irrigation
Site 1	38° 49' 16''	ORG	2.9	None	rainfed
	3° 16' 44''	CONV	3.4	None	rainfed
Site 2	38° 47' 42'' N	ORG	1.9	Metal posts	irrigated
	3° 9' 19'' W	CONV	2.1	None	rainfed
Site 3	38° 47' 29'' N	ORG	4.6	Metal posts	irrigated
	3° 9' 16''	CONV	5.7	None	rainfed

Table 2. Significance of the effect of sampling date (D, May 2013, October 2014, June 2014, October 2014), type of crop (C, olive groves or vineyards), management system (S, organic or conventional), and their interactions on the abundances (no. of nematodes/100g of dry soil) of nematode taxa found in the study area (**=p<0.05, ***=p<0.01). Mean nematode abundance (no. of nematodes / 100g dry soil) at each crop x system interaction is indicated. Trophic group of each taxa is shown (Ba=bacterivores, Fu=fungivores, H=herbivores, O=omnivores, P=predators, En=entomopathogen). Numbers within brackets next to nematode taxa are for nematode identification in Fig. 3.

| T.G. | Taxa | Date | Crop | System | D x C | D x S | C x S | Olive groves | | Vineyards | | D xC x S |
								CONV	ORG	CONV	ORG	
Ba	*Mesorhabditis*						***	0.54	0.37	0.05	0.20	**
Ba	*Rhabditis*							0.13	0.80	0.83	0.14	
Ba	*Cruznema*							0.00	0.00	0.00	0.04	
Ba	*Panagrolaimus*	***	***		***			23.99	25.37	18.79	9.35	
Ba	*Cervidellus*	***	**					15.12	12.16	8.66	7.46	
Ba	*Acrobeles*	***						7.73	7.46	7.93	5.33	
Ba	*Acrobeloides*	***	***		***		***	160.09	113.72	36.70	51.77	
Ba	*Metateratocephalus*							0.12	0.00	0.02	0.00	
Ba	*Teratocephalus*							0.12	0.00	0.00	0.00	
Ba	*Plectus*							0.43	1.85	0.43	0.07	
Ba	*Wilsonema*							0.25	0.26	0.06	0.16	
Ba	*Eumonhystera*							0.25	0.58	0.13	0.07	
Ba	*Chiloplacus*							0.17	0.00	0.00	0.00	
Ba	*Prismatolaimus*				**	**	***	2.57	3.95	2.05	0.47	**
Ba	*Achromadora*	***	***		***			3.40	1.51	0.00	0.00	**
Ba	*Alaimus*							0.01	0.00	0.02	0.36	
Fu	*Aphelenchus*	***	***		***			58.24	77.58	25.75	24.37	
Fu	*Aphelenchoides*	***	***		***			86.25	55.11	31.14	38.23	**
Fu	*Diphterophora*		**		**		**	0.06	0.00	0.01	1.05	
Fu	*Tylencholaimus*	***	***					0.89	1.51	0.01	0.39	

Table 2. Continuation.

T.G.	Taxa	Date	Crop	System	D x C	D x S	C x S	Olive groves		Vineyards		D xC x S
								CONV	ORG	CONV	ORG	
Fu	Tylenchidae		***	***	***			76.72	86.13	29.64	19.09	
Fu	*Aprutides*							0.39	0.70	0.00	0.00	
Fu	*Tylolaimophorus*							0.00	0.00	0.01	0.00	
H	*Ecphyadophora*							0.00	0.00	0.03	0.00	
H	*Tylenchorhynchus*	***	***	**	***			5.17	7.49	0.07	0.57	
H	*Paratylenchus*	***		***				7.52	21.36	1.79	8.93	
H	*Pratylenchus*	***	***	**				26.52	26.75	0.83	15.29	
H	*Meloidogyne*	***	***		***			0.17	0.14	3.73	12.49	
H	*Helicotylenchus*	***	***		***			9.95	16.13	0.35	1.70	
H	*Rotylenchus*	***	***		***			2.72	5.72	0.06	0.38	
H	*Trichodorus*							0.10	0.02	0.07	0.00	
H	*Xiphinema*	***	***	***	***			0.76	1.23	1.48	7.34	**
H	*Axonchium*							0.09	0.00	0.02	0.04	
H	*Paralongidorus*							0.04	0.00	0.00	0.00	
O	Qudsianematidae	***	***					9.69	12.32	3.00	5.27	
O	*Mesodorylaimus*							0.00	0.18	0.00	0.12	
O	Aporcelaimidae	**			***			0.55	0.06	0.27	0.47	***
P	*Tripyla*							0.00	0.17	0.00	0.00	
P	*Mylonchulus*							0.00	0.14	0.03	0.00	
P	*Clarkus*							0.00	0.10	0.00	0.00	
P	*Prionchulus*							0.00	0.07	0.00	0.00	
P	*Discolaimus*							0.24	0.36	0.11	0.08	
En	*Steinernema*							0.00	0.00	0.03	0.00	
Ba	Dauer							0.33	1.23	1.95	0.62	

Table 3. Significance of the effect of sampling date (D, May 2013, October 2014, June 2014, October 2014), type of crop (C, olive groves or vineyards), management system (S, organic or conventional), and their interactions on soil food web descriptors (MI= Maturity Index, ∑MI= Sigma Maturity Index, PPI= Plant-Parasitic Index, CI= Channel Index, BI= Basal Index, EI= Enrichment Index, SI= Structure Index, Biom= nematode biomass (µg/g soil), Tot= total nematode abundance (No. nematodes/100g dry soil), H'= Shannon diversity Index; S= Taxa richness). F-statistic and significance of the p-value is indicated. Mean ± SE nematode community descriptors in the four systems is shown.

	Date		Crop		System		D x C		D x S		C x S		Olive groves		Vineyards		D x C x S	
	F	p	F	p	F	p	F	p	F	p	F	p	CONV	ORG	CONV	ORG	F	p
MI	28.9	<0.01	0.05	ns	1.83	ns	4.44	<0.01	1.46	ns	2.70	ns	2.06±0.02	2.06±0.02	2.03±0.03	2.09±0.03	3.53	<0.05
∑MI	26.3	<0.01	0.96	ns	6.31	<0.05	3.85	<0.05	0.15	ns	4.65	<0.05	2.13±0.02	2.15±0.03	2.11±0.04	2.25±0.5	1.48	ns
PPI	12.18	<0.01	3.89	<0.01	3.96	<0.05	5.76	<0.01	0.87	ns	2.86	ns	2.30±0.03	2.32±0.04	2.34±0.07	2.54±0.08	0.99	ns
CI	12.4	<0.01	2.01	ns	0.05	ns	2.47	ns	1.34	ns	4.46	<0.05	66.2±3.3	60.1±3.3	57.9±3.5	61.2±3.7	3.25	<0.05
BI	8.72	<0.01	7.20	<0.01	0.03	ns	0.21	ns	0.21	ns	8.49	<0.01	53.2±1.9	48.0±1.7	44.4±1.6	48.1±2.0	1.26	<0.05
EI	3.70	<0.01	8.72	<0.01	0.04	ns	2.81	<0.05	0.55	ns	15.23	<0.01	37.1±1.9	43.1±1.7	48.0±1.6	42.0±1.9	2.50	ns
SI	33.9	<0.01	0.39	ns	0.24	ns	2.63	ns	1.00	ns	0.01	ns	19.0±2.3	21.1±2.5	20.1±2.5	22.2±3.2	2.11	ns
Biom.	2.56	<0.01	3.57	ns	2.47	ns	3.02	ns	1.31	ns	2.09	ns	0.22±0.0	0.24±0.0	0.28±0.1	0.79±0.3	1.19	ns
Tot	8.75	<0.01	84.58	<0.01	0.14	ns	10.42	<0.01	1.93	ns	0.55	ns	505.9±50.0	480.6±43.4	170.0±20.8	213.9±21.9	2.41	ns
H'	17.92	<0.01	0.14	ns	0.20	ns	0.07	ns	1.84	ns	8.26	<0.01	1.70±0.04	1.81±0.03	1.81±0.04	1.70±0.04	1.11	ns
S	14.32	<0.01	11.73	<0.01	0.84	ns	5.76	ns	1.53	ns	2.19	ns	10.4±0.3	11.1±0.3	9.9±0.3	9.8±0.03	1.52	ns