

# *Interacting effects of land use type, soil microbes and plant traits on aggregate stability*

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1 **Interacting effects of land use type, soil microbes and plant traits on aggregate stability**

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19

## 20 **Summary**

21 Soil aggregates are critical to soil functionality, but there remain many uncertainties with respect to  
22 the role of biotic factors in forming aggregates. Understanding the interacting effects of soil, land use  
23 type, vegetation and microbial communities is a major challenge that needs assessment in both field  
24 and controlled laboratory conditions, as well as in bulk and rhizosphere soils. To address these effects  
25 and their feedbacks, we first examined the influence of soil, root and litter characteristics along a land  
26 use gradient (ancient woodland, secondary woodland, grassland, pasture and arable land) on microbial  
27 community structure (in both bulk and rhizosphere soil), as well as on aggregate stability. Then, we  
28 performed an inoculation experiment where we extracted soil columns from the arable and secondary  
29 woodland and used a third unstructured loamy soil as a control. We sterilized these three soils to  
30 remove microbial communities, and then either inoculated the tops of sterilized soil columns with soil  
31 from the secondary woodland or the arable field sites. Control columns of all soil types were not  
32 inoculated. In a fully-crossed design, we planted two species possessing distinct root system  
33 morphological traits: *Brachypodium sylvaticum* (fibrous system with many thin and fine roots) and  
34 *Urtica dioica* (taproot system with few fine roots). After four months, microbial communities (in bulk  
35 and rhizospheric soil) and aggregate stability were measured, along with root traits. In both the field  
36 and laboratory experiments, bacterial (16S) and fungal (ITS) biodiversity was determined using high  
37 throughput sequencing. In the field study we found that: i) there were strong relationships between  
38 aggregate stability and microbial community composition that were driven by land use, ii) the  
39 relationship between aggregate stability along the land use gradient and the trophic nature of bacterial  
40 communities was not significant, but that certain soil, root and litter parameters shaped bacterial  
41 phyla, with oligotrophic bacteria conditioned by the rhizosphere niche, and copiotrophic phyla more  
42 dependent on bulk soil conditions, iii) land use gradient (from woodland to arable), reduced the  
43 relative abundance of saprotrophic and ectomycorrhizal fungi with an increase in the relative  
44 abundance of Ascomycota and a reduction in the relative abundance of Basidiomycota. In the  
45 laboratory experiment we found that: i) the inoculation of sterilized soils with soils from the field  
46 significantly increased aggregate stability in control soil that was initially poorly structured, ii) the  
47 effects of inoculation on aggregate stability were similar when either secondary woodland or arable

48 soils were used as inoculums and iii) these effects were affected significantly by root length density.  
49 Our results show that microbial communities influence soil structure and that bacterial communities  
50 are intimately associated to rhizospheric conditions and root traits (of which root length density was  
51 the most pertinent).

52 **Key words:** bacteria, fungi, glomalin, ergosterol, ITS, root traits, root systems, soil structure, 16S.

## 53 **1. Introduction**

54 Soil structure is an essential factor driving the success of many ecosystem services worldwide  
55 (Adhikari and Hartemink, 2016), but most soils have been severely degraded in recent decades, due  
56 largely to anthropogenic pressures (Jie et al., 2002; Peng et al., 2015). Therefore, the capacity of soil  
57 to retain water, maintain biodiversity, sustain agriculture and resist flooding, erosion and landslides is  
58 compromised, unless we can find mechanisms by which soil structure can be restored rapidly. Soil is  
59 composed of a solid phase of particles and a pore phase that provides pathways for the transport of  
60 water, nutrients and gases, as well as habitats for microorganisms and fauna. The spatial configuration  
61 of soil particles and pores is arranged in aggregates and their stability is an important aspect of soil  
62 structure. Apart from soil texture (Bissonnais and Arrouays, 1997), diverse biotic and abiotic factors  
63 drive the rate of aggregation and its stability, e.g. microorganisms (Tisdall, 1994; Chenu and Sotzky,  
64 2002; Lehmann et al., 2017), fauna (Lee and Foster, 1991; Ayuke et al., 2011), litter and root traits  
65 (Baumert et al., 2018; Poirier et al., 2018), environmental variables (e.g., tillage, freeze-thaw, wetting  
66 and drying cycles) (Lavee et al., 1996; Six et al., 1998; Gispert et al., 2013b) and inorganic binding  
67 agents (e.g., clay minerals, oxides and polyvalent cations such as calcium) (Denef and Six, 2005).  
68 Although many studies have focused on how individual factors modify soil aggregation and stability,  
69 very few have considered combinations of driving factors and their effects (Six et al., 2004; Baumert  
70 et al., 2018), particularly complex biotic factors. However, a better understanding of these interactions  
71 will improve soil restoration and the production of ecosystem services (Lavelle et al., 2020).

72 The importance of biotic factors for soil aggregation, and in particular soil microorganisms, was first  
73 suggested by Tisdall (1994) and has since been recognized worldwide (e.g. Degens, 1997; Chotte,  
74 2005; Gupta and Germida, 2015). In a global meta-analysis, Lehmann et al. (2017) found a positive  
75 effect of soil biota on aggregation, with bacteria and fungi being generally more important for  
76 aggregation than other groups. Good soil structure is usually associated with high microbial biomass  
77 (Degens, 1997) and activity (Cui and Holden, 2015), mainly through the chemical binding action of  
78 microbial compounds such as bacterial extracellular polymeric substances (EPS) or soil proteins, or  
79 through the physical enmeshment of soil particles by fungal hyphae (Oades and Waters, 1991; Rillig

80 and Mummey, 2006). Rillig et al. (2002a,b) attributed the positive effect of plant roots on aggregation  
81 to the excretion of a protein by arbuscular mycorrhizal fungi (AMF) called glomalin. This protein is  
82 operationally defined by its extraction method and belongs to a group of proteins after called  
83 glomalin-related soil proteins (GRSPs) that are no longer believed to be solely of fungal origin.  
84 Ergosterol is the most abundant sterol in fungal cell membranes and since fungal hyphae enmeshment  
85 is a major factor for the formation of macroaggregates, it should be related to aggregate stability. Yet,  
86 the feedback mechanisms between aggregate formation and stability and the influence of microbial  
87 communities are not fully understood and identifying the relationships between changes in microbial  
88 community structure and GRSP and ergosterol are key for understanding soil aggregation processes.

89 Root system morphology can influence soil aggregation through changes in the distribution of carbon  
90 inputs into soil (Carter et al., 1994; Degens, 1997). Fine roots have direct effects on macroaggregate  
91 stability through the production of mucilage and exudates and indirect effects through the promotion  
92 of hyphal growth and the release of root-derived particulate organic matter (POM) that stimulate  
93 microbial activity (Morel et al., 1991; Miller and Jastrow, 1992; Brax et al., 2020). Consequently,  
94 plants with fibrous root systems, that have numerous, evenly distributed thin and fine roots, may have  
95 more available mucilage/exudates, as well as more easily degradable POM, than plants with taproot  
96 systems. Taproot systems generally comprise one large central root and less thin or fine roots (Miller  
97 and Jastrow, 1992; Degens, 1997), thus the potential input of total carbon into soil is reduced. We ask  
98 therefore, if root system morphology, and individual root traits such as diameter, length and carbon  
99 and nitrogen content (Saleem et al., 2018), influence the composition of microbial communities and  
100 so can modify macroaggregate formation and stability.

101 Soil aggregates are important for microbial community ecology, evolution and for microbially  
102 mediated cycles (Goebel et al., 2009; Rillig et al., 2016; Rillig et al., 2017). The heterogeneity of  
103 pores and water films on soil particles leads to a diversity of microhabitats and gradients of abiotic  
104 traits (e.g. nutrients and pH) at microscales (Or et al., 2007). Soil carbon type and availability  
105 provides strong selective pressures for the different lifestyle strategies among copiotrophs (common  
106 in carbon-rich environments) and oligotrophs (present in habitats of low carbon flux) (Fierer et al.,

107 2007; Eilers et al., 2010; Trivedi et al., 2017). Trivedi et al. (2017) showed that aggregate size  
108 modulated the effects of management practices on soil carbon, with oligotrophs (e.g. Acidobacteria,  
109 Chloroflexi and Verrucomicrobia) inhabiting microaggregates containing higher proportions of  
110 recalcitrant carbon and copiotrophs (e.g.  $\alpha$  and  $\beta$  Proteobacteria, Bacteroidetes and Actinobacteria)  
111 living in macroaggregates with more labile carbon. Hence, soils with high macroaggregate stability  
112 should be dominated by copiotrophs. However, aggregate size is not the only factor influencing the  
113 abundance of copiotrophs and oligotrophs. Lauber et al. (2009) and Carbonetto et al. (2014) showed  
114 that agro-systems had a higher abundance of copiotrophs than in forests, probably because of greater  
115 nitrogen fertilization stimulating copiotrophic communities (Fierer et al., 2012). Complex interactions  
116 in soil can influence the abundance of different lifestyle strategies, e.g., rhizosphere communities are  
117 enriched with rhizodeposits (Dennis et al., 2010) and as a consequence, increase copiotroph  
118 abundance compared to bulk soil (Peiffer et al., 2013; Lladó and Baldrian, 2017). However, studies on  
119 how root and litter traits affect the abundance of these trophic modes in the different soil niches are  
120 notably lacking (Saleem et al., 2018), even though these scaled down studies are still needed to  
121 decipher the rules of rhizosphere community assembly (Brunel et al., 2020). The disturbance of soils  
122 through management also decreases soil fungal diversity, increasing the prevalence of generalist taxa,  
123 reducing the relative abundances of saprotrophic and ectomycorrhizal fungi and increasing the  
124 abundance of pathogenic fungi (Mueller et al., 2016; Marín et al., 2017). Managed land use types also  
125 possess more Ascomycota and less Basidiomycota compared to undisturbed natural ecosystems (de  
126 Castro et al., 2008; Mueller et al., 2016). Here, we ask if differences in the abovementioned bacterial  
127 and fungal trophic modes are due mainly to differences in aggregate formation, soil chemical  
128 characteristics or plant traits, along a land use gradient.

129 Since the complex feedbacks between aggregate formation and microbial communities are not fully  
130 understood, studies that integrate field observation with experimental testing are needed. Here, we  
131 performed two studies to determine the combined effects of soil type, root system morphological traits  
132 and microbial communities on soil aggregation and stability. We first surveyed these responses in the  
133 field across a gradient of different land use types (grassland, pasture, arable land, secondary woodland



134 and ancient woodland) and hypothesized (hypothesis 1) that there are strong relationships between  
135 soil aggregate stability and microbial community structure that are driven by land use. Additionally,  
136 we hypothesize (hypothesis 2) that soils of higher aggregate stability will have characteristic  
137 communities comprising increased bacterial copiotrophs and increased relative abundance of  
138 saprotrophic and ectomycorrhizal fungi related to a decrease in the relative abundance of Ascomycota  
139 and an increase of Basidiomycota. However, this relationship may be influenced by other edaphic  
140 factors (e.g. soil nitrogen content) and root and litter traits, or soil habitat (e.g., with the rhizosphere  
141 niche favouring copiotrophic bacterial communities).

142 Then, to experimentally test interactive effects of plants and microbes on aggregate stability, we  
143 performed a controlled experiment where we sterilized soil samples from arable land and secondary  
144 woodland as well as a control soil; and inoculated into sterilized soil in which was grown two species  
145 of herbaceous plants with different root system morphologies. We hypothesize (hypothesis 3) that  
146 very fibrous root systems will increase aggregate stability, because more labile carbon will be  
147 available for microbial communities from fine root exudates and mucilage. We further hypothesize  
148 (hypothesis 4) that the effect of inoculation on aggregate stability will be greater with the inoculum  
149 coming from the soil with highest aggregate stability (secondary woodland), and this is related to  
150 source microbial community characteristics (measured using sequencing, GRSP, fungal biomass  
151 ergosterol ).

## 152 **2. Material and methods**

### 153 **2.1. Field sampling**

154 The study site is located in Wytham, Oxfordshire, southern England (1° 20 ' W, 51° 47 ' N), where  
155 three land uses (grassland, pasture and arable) and two forests (ancient woodland and secondary  
156 woodland) were selected (Fig. 1). The forests are located in a 410 ha area of mixed woodlands and  
157 grassland, on a hill owned by the University of Oxford since 1943. These forests have been studied  
158 for several decades (Watts, 1969; Mihók *et al.*, 2009) and the management history of the site is well  
159 documented (Gibson, 1986). In this area, we selected two forests for our study, an ancient woodland

160 and a secondary woodland. The ancient woodland was traditionally managed by coppicing, but this  
161 management stopped between 40 and 100 years ago (differing locations were abandoned at different  
162 times). *Acer pseudoplatanus* L. and *Quercus robur* L. are abundant and *Corylus avellana* L. was the  
163 main coppice species. The dominant tree species of the naturally regenerated secondary woodland are  
164 *Fraxinus excelsior* L. and *Acer pseudoplatanus* L. (Savill et al., 2011). The ground flora of these  
165 woodlands is characterised by dominant *Mercurialis perennis* (dog's mercury) and changeable  
166 combinations of *Endymion non-scripta* (bluebell), *Circaea lutetiana*, *Geum urbanum*, *Arum*  
167 *maculatum* and *Viola riviniana/reichenbachiana*. *Rubus fruticosus* and bryophytes are common  
168 features of the field layer, in addition to the sedge *Carex sylvatica* and the grasses *Poa trivialis* and  
169 *Brachypodium sylvatica* (Butt et al., 2009). The three other land uses that were selected for this study  
170 are in an adjacent farmland. The arable site was cultivated with winter oats (*Avena sativa* L.) with no  
171 fertilization in 2014 (the sampling year) and with spring wheat (*Triticum aestivum* L.), fertilized at a  
172 rate 164 kg N ha<sup>-1</sup> and 7 t ha<sup>-1</sup> of pig manure in 2013. In this arable site, soil was ploughed prior to our  
173 sampling. The other two sites, grassland and pasture, have been permanent pastures since 1992, with  
174 the only difference that the grassland had not been actively grazed the year preceding the study (2013-  
175 2014), increasing drastically the aboveground biomass of vegetation. Soils in the area span a  
176 geological sequence from Oxford Clay to Upper Corallian sand and silt to Upper Corallian limestone  
177 and Coral Rag developing soils with characteristic textures (Savill et al., 2011). All sites selected for  
178 this study are located on the Oxford Clay formation with Denchworth series soil (Eutric Vertic  
179 Stagnosols *sensu* USS Working Group WRB (2014)).

180 We collected soil samples to examine relationships in soil properties and microbial communities  
181 between sites and to provide background data for the inoculation experiment (see below). In each land  
182 use type, sampling was performed in three plots that were randomly located and samples were  
183 collected at a depth of 0-0.1 m except for aggregate stability samples and soil physicochemical  
184 properties that were collected at two depths (0-10 cm and 20-30 cm). Depth can influence aggregate  
185 stability as it affects soil properties that are related to soil aggregate stability such as total organic  
186 carbon, total nitrogen, freeze-thaw cycles, etc. Four different soil samples were collected at each

187 depth using sterilised material: (i) one soil cylinder ( $1.01 \text{ dm}^3$ ) for fine root analysis, (ii) 10 to 20 g of  
188 soil for microbial analysis placed in a plastic bag, (iii) soil for measurements of aggregate stability (at  
189 depths of 0-0.1 m and 0.2-0.3 m) placed in  $0.75 \text{ dm}^3$  containers and (iv) two soil samples for soil  
190 physicochemical analyses (at depths of 0-0.1 m and 0.2-0.3 m). Litter from the surface of each soil  
191 core was sampled and kept in plastic bags for further analysis. A total of 15 samples of each type were  
192 collected: five land uses x three samples at each land use (except for aggregate stability and soil  
193 physicochemical properties where a total of 30 samples were collected, since six samples were  
194 collected at each plot; three at 0-0.1 m and three at 0.2-0.3 m). Soil samples were collected during  
195 July 7th - 10th, 2014, at the peak of the summer season when microbial activity was expected to be at  
196 its maximum (Sarithchandra et al., 1988; Bardgett et al., 1997). Soil physicochemical properties of  
197 the five sites are given in Table 1.

198 Samples for root, litter and microbial analysis were stored at  $-20^\circ\text{C}$  prior to analysis. Soil samples for  
199 aggregate stability tests were air-dried at ambient temperature and analysed within one month.

## 200 **2.2. Laboratory inoculation experiment setup and sampling**

201 Intact soil cores with a diameter of 110 mm and a length of 150 mm ( $1.42 \text{ dm}^3$ ) were collected from  
202 two field sites, arable land and secondary woodland in close proximity (300 m) for the laboratory  
203 inoculation experiment (Fig. 2). These two land uses were selected based on their large differences in  
204 soil aggregate stability and the minimal differences in soil textural properties (Table 1, Fig. 3). Thirty-  
205 three intact soil cores in the arable land site were collected on January 7<sup>th</sup>, 2015 and another set of 33  
206 soil cores were collected in Wytham woods on January 8<sup>th</sup>, 2015 (Figs. S1 and S2a). Thirty-three pots  
207 were also filled with a commercial loamy soil (Boughton Kettering Loam, Boughton Loam Ltd, U.K.)  
208 to be used as a control treatment because this loamy soil lacks structural stability. Before and after  
209 sterilization, three samples from each provenance were collected for the measurement of  
210 physicochemical properties (Table S1).

211 After the field collection, the upper 30 mm of each soil core were removed to allow subsequent  
212 inoculation with 15 mm of non sterile soil from the arable and woodland soils ( $0.14 \text{ dm}^3$ , 10% of total

213 volume) and 15 mm free for water ponding from irrigation (Figs. S2b and c). Thirty soil cores of each  
214 treatment (arable, woodland and control) were sterilized by gamma irradiation at Elgin Synergy  
215 Health PLC (Swindon, U.K.) with a dose between 50.5 and 52.1 kGy on January 29<sup>th</sup>, 2015. Three  
216 cores of each soil were used to assess aggregate stability after gamma radiation and compared with  
217 the three non sterilized cores to verify that there was no effect of gamma irradiation on aggregate  
218 stability. Similarly, one soil sample in each of these three cores was collected to assess changes in  
219 microbial communities before and after the gamma radiation (three samples assessed before and three  
220 samples after).

221 After irradiation, the upper 15 mm of 18 soil cores was inoculated with fresh soil collected on  
222 February 9<sup>th</sup>, 2015 from the arable and woodland fields (nine soil cores from each provenance) and  
223 another set of nine soil cores were not inoculated (controls).

224 In three replicates of each combination, seeds from two species with either tap/adventitious (*Urtica*  
225 *dioica*) or fibrous (*Brachypodium sylvaticum*) root systems (*sensu* Fitter and Peat, 1994) were sown  
226 and a third treatment with no plants was used as a control (see Fig. 2 and Fig. S2 for a detailed  
227 illustration of procedure). Seeds of the two selected species, abundant in the Wytham area with  
228 contrasting root systems (Savill et al., 2011), were obtained from two different suppliers: Emorsgate  
229 Seeds, U.K. (*B. sylvaticum*) and Naturescape British Wildflowers, U.K. (*U. dioica*). Seeds were  
230 sterilized by placing them in a mesh bag and left for 30 min in a 2% sodium hypochlorite solution.  
231 They were removed from the bag and rinsed three times (for 5 min each) in sterilized deionised water  
232 (Commander et al., 2009). Then, seeds were germinated for three weeks in sterilized, deionised water  
233 before sowing. Ten seeds of each species were sown in each pot. Plants were grown in a temperature  
234 and light controlled facility for 4 months (February 12<sup>th</sup> to June 15<sup>th</sup>, 2018, Fig. S2d). Pots were  
235 regularly weeded by hand for the first six weeks, but after that, the species that were sown  
236 commenced growth and weeding was ceased to avoid disturbance, although some seedlings emerged  
237 (particularly in treatments inoculated with arable soil).

238 The first three irrigation events were performed with autoclaved deionised water, but afterwards, pots  
239 were watered with tap water using an automatic station (to promote downward movement of  
240 inoculum). To identify microbial communities existing in tap water and subtract them from the later  
241 microbial analysis on soils, 5 l of tap water were collected at the beginning and the end of the  
242 experiment. This water was filtered, and DNA was extracted.

## 243 **2.3. Sample analysis**

### 244 **2.3.1. Soil respiration, moisture and physicochemical properties**

#### 245 **2.3.1.1. Field study**

246 Soil collected in the field study was sieved at 2 mm after air drying and the physicochemical  
247 properties of the < 2 mm soil fraction was quantified by Natural Resource Management (Berkshire,  
248 UK). Soil pH was measured in water as 1:2.5 extract. Soil organic matter content (SOM) was  
249 determined via loss-on-ignition at 500 °C (Dean 1974). Total nitrogen (N) and carbon (C) were  
250 determined via the DUMAS method (Shea and Watts 1939). Available potassium (K) and magnesium  
251 (Mg) were determined through ammonium nitrate extraction and available phosphorus (P) was  
252 measured using the Olsen method (extraction with 0.5 M sodium bicarbonate solution, (Olsen et al.  
253 1954)). Soil texture was determined by laser-diffraction analysis (McCave et al. 1986). Each soil  
254 sample was previously digested in hydrogen peroxide solution to destroy the organic matter and  
255 sodium hexametaphosphate to release the bound clay particles.

256 Aggregate stability was determined by the fast wetting standard method, ISO/CD 10930, developed  
257 by Le Bissonnais (Le Bissonnais, 1996). This methodology is appropriate to compare the behaviour of  
258 a large range of soils during rapid wetting (mimicking heavy rainstorms in summer). A quantity of 5 g  
259 of aggregates (3-5 mm) were gently immersed in 50 ml of deionized water for 10 minutes; water was  
260 then removed with a pipette and the soil material was transferred to a 50 µm sieve previously  
261 immersed in ethanol. The 50 µm sieve immersed in ethanol was gently moved five times to separate  
262 fragments smaller and bigger than 50 µm. The >50 µm fraction was collected, oven-dried and gently

263 dry-sieved by hand on a column of six sieves: 2000, 1000, 500, 200, 100 and 50  $\mu\text{m}$ . The mass  
264 percentage of each size fraction was calculated, and the aggregate stability was expressed by  
265 computation of the mean weight diameter (MWD).

#### 266 **2.3.1.2. Laboratory inoculation experiment**

267 Soil samples collected from the inoculation experiment were sent to the chemical analysis laboratory  
268 of Forest Research (Surrey, UK). Soil pH was measured in a suspension of 5 g of soil with 25 ml of  
269 water that was shaken on an orbital shaker for 15 min and settled for 45 min prior to pH measurement  
270 using a Sentek pH electrode (Reference method: ISO 10390). Total N, C, soil organic carbon (SOC)  
271 and inorganic carbon ( $\text{CaCO}_3$ ) were measured with a combustion method using a Carlo Erba CN  
272 analyser (Reference method ISO 10694 & 13878). Aggregate stability was measured with the fast  
273 wetting standard method described above.

274 We examined if microbial inoculation (through the addition of surface soil) changed soil texture and  
275 aggregate stability in deeper soil. The top 15 mm of soil was removed, and aggregate stability  
276 measured in the remaining soil. Texture of the aggregates obtained at the end of the experiment was  
277 measured by the CIRAD - US Analyses (Montpellier, France). The objective was to test whether the  
278 texture of the obtained aggregates was more related to the inoculated core (origin) or to the *inoculum*  
279 (inoculation treatment) and so, if there was any possible influence of inoculation with soil on the  
280 results of stability tests. Soil texture of aggregates was determined with an automated pipette method  
281 (Reference method AFNOR NF X 31-107) using a Texusol granulometer (ISITEC-LAB) on samples  
282 pre-treated following the standard method NF ISO 11464 (X31-412).

283 Two additional measurements in soils were made: i) the rate of exchange of  $\text{CO}_2$  (assimilation; g  
284 ( $\text{CO}_2$ )  $\text{m}^{-2} \text{h}^{-1}$ ) measured with an EGM-4 Environmental Gas Monitor for  $\text{CO}_2$  and a SRC-1 Soil  
285 Respiration Chamber (PP Systems, Amesbury, MA, U.S.A.) in June 15th-17th, 2015 and ii) soil water  
286 content measured at two dates (June 8th and 12th, 2015) after irrigation with a WET Sensor (Delta-T  
287 Devices Ltd, Cambridge, UK).

### 288 2.3.2. Aboveground vegetation, fine roots and litter

289 In the soil inoculation experiment, all aboveground vegetation biomass was cut at the surface at the  
290 end of the experiment and dried at 60°C until constant weight (Fig. S2e). Then, a core (0.295 dm<sup>3</sup>)  
291 was collected in each of the pots for the assessment of root properties (Fig. S2f and Fig. S3).

292 Root samples from both the field study and the inoculation experiment were washed gently with  
293 deionised water and divided in two subsamples: 1) a representative subsample that was selected for  
294 scanning and later drying in the oven at 40°C (n=3 for each land use in the field experiment and n =  
295 27 for each provenance; arable, woodland and control; in the inoculation experiment) and 2)  
296 remaining root material of the sample that was dried at 40°C until constant weight (n=3 for each land  
297 use in the field experiment and n = 27 for each provenance in the inoculation experiment). Both  
298 subsamples were weighed before and after drying. Roots selected for morphological measurement  
299 were stained with methylene blue (1 gl<sup>-1</sup>) to increase the contrast and allow the detection of fine roots.  
300 Then, roots were placed in a tray with deionised water and scanned (Epson© V700 Perfection) at a  
301 resolution of 1200 dpi. Analysed subsample roots were then recovered, and oven dried at 40 °C and  
302 weighed to obtain dry mass. Root images were analysed with the WinRhizo® software (Pro version  
303 2007, Regent Instrument, Quebec, Canada) using the automatic thresholding option and Lagarde's  
304 mode, with a filter identifying roots when length was five times the width. We measured total root  
305 length and the length of roots in seven diameter classes (roots with a width of 0 - 1 mm were  
306 separated into 0.2 mm classes and roots with a width of 1 - 2 mm were separated into 0.5 mm classes).  
307 Debris was removed with a filter that eliminated fragments that were less than five times longer than  
308 their width. Specific root length (SRL) was calculated as the ratio between total root length and root  
309 dry mass. The percentages of very fine (VFR, diameter < 0.2 mm) and fine (FR, 0.2 < diameter < 1  
310 mm) roots were defined as the ratio of length in the concerned root classes to total root length (Miller  
311 and Jastrow, 1990). Total root mass density (RMD) was calculated by the ratio of total root dry mass  
312 and the soil volume extracted. Total root length density (RLD) was calculated by the ratio of total root  
313 length and the soil volume extracted. Total root dry mass was calculated as the sum of the dry mass of

314 roots selected for morphological analysis and those of the remaining roots. Root dry matter content  
315 (RDMC) was calculated as the ratio of the root dry weight and fresh weight.

316 The concentrations of water soluble compounds (cellulose, hemicellulose and lignin;  $\text{mg g}^{-1}$ ) in root  
317 and litter samples from the field experiment ( $n = 11$  for litter, i.e. 3 replicates x 4 treatments = 12  
318 excluding 1 sample with insufficient mass; and  $n = 4$  for roots as replicates were combined due to  
319 limited sample amount; “arable” was excluded from these measurements), were obtained by the Van  
320 Soest Method (Van Soest, 1963) with a Fibersac fibre analyser (Ankom, Macedon, USA). Root C and  
321 N concentrations were measured in roots from the field study ( $n=12$ , i.e. 3 replicates x 4 treatments  
322 since arable was excluded from these measurements) and the inoculation experiment ( $n = 3$  for each  
323 treatment with roots) and litter C and N were measured in samples from the field experiment ( $n=15$ )  
324 using an elemental analyser (Thermo-Finnigan EA1112, Italy).

### 325 **2.3.3. Soil microbial communities**

#### 326 **2.3.3.1. Obtaining rhizosphere and bulk soil samples**

327 In both the field study and laboratory inoculation experiment, roots were cleaned and rhizosphere and  
328 bulk soil samples obtained following the protocols of Bulgarelli et al. (2012, 2015). Briefly, loose soil  
329 was manually removed from the root system and stored as the bulk soil sample. Roots were collected  
330 in 50 ml falcon tubes containing 10 ml PBS-S buffer (130 mM NaCl, 7 mM  $\text{Na}_2\text{HPO}_4$ , 3mM  
331  $\text{NaH}_2\text{PO}_4$ , pH 7.0, 0.02 % Silwet L-77) and washed for 20 minutes at 180 rpm on a shaking platform.  
332 These roots were transferred to a new falcon tube and subjected to a second washing treatment (20  
333 minutes at 180 rpm in 3 ml PBS-S buffer). The soil suspensions collected in the falcon tubes after the  
334 first and second washing treatments were combined, centrifuged at 4000 g for 20 min and the pellet,  
335 considered as the rhizosphere sample, was frozen and stored at  $-20^\circ\text{C}$  until further analysis.

#### 336 **2.3.3.2. DNA extraction, amplicon library construction and sequencing**

337 Total DNA was extracted from soil (0.25 g) and the rhizosphere fractions (0.25 g when possible and  
338 the entire material available when quantity was less than 0.25 g). DNA extraction was performed



339 using PowerSoil®-htp96 Well Soil DNA Isolation Kit according to the manufacturer's instructions  
340 (MOBIO Laboratories, UK).

341 Bacterial and fungal community biodiversity was assessed using Illumina amplicon sequencing of  
342 16S rRNA genes (bacteria) and the Internal transcribed spacer (ITS) region (fungi) to  
343 phylogenetically identify responsive taxa. A phylogenetic analysis was also performed for bacterial  
344 communities. Amplicon libraries were constructed according to the dual indexing strategy of Kozich  
345 et al. (2013), with each primer consisting of the appropriate Illumina adapter, an 8-nt index sequence,  
346 a 10-nt pad sequence, a 2-nt linker and the gene specific primer. For 16S, the V3-V4 hypervariable  
347 regions of the 16S rRNA gene was targeted using primers based upon the universal primer sequence  
348 341F and 806R. For ITS, region 2 (ITS2) was amplified utilising the fITS7 (forward) and ITS4  
349 (reverse) primer sequences described in Ihrmark et al. (2012). Additional methodological details of  
350 Illumina sequencing are described in Notes S1.

#### 351 **2.3.3.3. Processing high-throughput data for community analysis**

352 Sequenced 16S rRNA paired-end reads were joined using PEAR (Zhang et al., 2014), quality filtered  
353 using FASTX tools (hannonlab.cshl.edu), length filtered with the minimum length of 300 bps,  
354 presence of PhiX and adapters were checked and removed with BBTools (jgi.doe.gov/data-and-  
355 tools/bbtools/), and chimeras were identified and removed with VSEARCH\_UCHIME\_REF (Rognes  
356 et al., 2016) using Greengenes Release 13\_5 (at 97%) (DeSantis et al., 2006). Singletons were  
357 removed and the resulting sequences were clustered into operational taxonomic units (OTUs) with  
358 VSEARCH\_CLUSTER (Rognes et al., 2016) at 97% sequence identity (Tindall et al., 2010).  
359 Representative sequences for each OTU were taxonomically assigned by RDP Classifier with the  
360 bootstrap threshold of 0.8 or greater (Wang et al., 2007) using the Greengenes Release 13\_5 (full)  
361 (DeSantis et al., 2006) as the reference. Unless stated otherwise, default parameters were used for the  
362 steps listed. ITS2 sequences were processed using the PIPITS pipeline (Gweon et al., 2015), where  
363 OTUs were taxonomically assigned against the UNITE database (Release 31.01.2016, Koljalg et al.  
364 (2013).

#### 365 **2.3.4. Glomalin-Related Soil Proteins (GRSP).**

366 Hurisso et al. (2018) recently proposed the term autoclaved-citrate extractable protein (ACE), to avoid  
367 the implied assumption to the fungal origin of these proteins, but here we use the term Glomalin-  
368 Related Soil Proteins (GRSP) for ease of comparison with other studies. In the inoculation  
369 experiment, soil samples from the middle of each soil core were collected after the incubation period  
370 for the assessment of GRSP, thought to be a marker of arbuscular mycorrhizal activity. Soil samples  
371 were stored at -20 °C until further processing. Operationally-defined GRSP fractions were obtained  
372 using the extraction methods proposed by Wright and Upadhyaya (1996) as follows. Easily  
373 extractable soil protein (GRSP<sub>EE</sub>) was obtained by autoclaving soil in a solution of 20 mM sodium  
374 citrate at pH 7 for 30 min. Total GRSP (GRSP<sub>T</sub>) was obtained by two successive autoclave extractions  
375 of soil in 50 mM sodium citrate at pH 8, each for 60 min. In each case, the soil:solution ratio was 1:8  
376 and phases were separated after cooling by centrifugation at 15000 g for 15 min. For GRSP<sub>T</sub>, after the  
377 first autoclave cycle, solution was removed and replaced by the same volume of new addition of  
378 citrate solution, the mixture was vortexed to re-suspend the soil. The solutions were combined to form  
379 the total extract. Solutions were frozen until required for analysis. After thawing, samples were  
380 centrifuged again at 15000 g for 15 minutes to remove any precipitate that may have formed. Protein  
381 in both extracts were assayed using the Bradford technique with Bradford QuickStart kits from  
382 BioRad Laboratories (Hercules, CA, USA), calibrated against solutions of bovine serum albumin  
383 (BSA) within the working range of 0-200 mg dm<sup>-3</sup>. Following the recommendations proposed by  
384 Moragues-Saitua and co-workers (Moragues-Saitua et al., 2019), samples were diluted twofold  
385 (absorbance of about 0.1 at 465 nm), to reduce the interference of co-extracted coloured compounds.  
386 A sample volume of 20 µl were assayed in microplates with 230 µl Bradford dye reagent. Optical  
387 density was measured at 595 nm in ThermoScientific Multiskan GO spectrometer (Waltham, MA,  
388 USA) and sample blank colour absorbance at pH=1 corrected.

#### 389 **2.3.5. Ergosterol**

390 Ergosterol, a proxy for active fungal biomass, was measured in soil samples that were obtained from  
391 the middle of each core of the inoculation experiment after the incubation period. Ergosterol was  
392 assessed following the method of Plassard et al. (2000). Briefly, ergosterol was extracted by mixing  
393 very gently 0.5 g of soil in 3 ml of methanol containing polyclar (0.5%, w/v) (CAS n° 9009-39-8) in  
394 screw-cap tubes. After firm closure, tubes were incubated at 4°C for 24 h, without shaking as  
395 ergosterol is rapidly lost by oxidation. The concentration of ergosterol in filtered (0.45 µm) methanol  
396 extracts was determined at 270 nm by high-performance liquid chromatography using a C18 column  
397 and eluted with methanol flowing at 1 ml min<sup>-1</sup>.

## 398 **2.4. Statistical analysis**

### 399 **2.4.1. Field study**

400 First, we examined the differences on soil, root and litter physicochemical characteristics and  
401 aggregate stability among the different land uses. Then, we performed NMDS data ordinations of  
402 microbial data and tested of the effect of land use and sample fraction on their structure  
403 (Permutational Multivariate Analysis of Variance, PERMANOVA). Finally, we studied the  
404 correlation between microbial community composition and environmental variables (Spearman  
405 correlations between Shannon diversity indexes and distance-based redundancy analysis, dbRDA).

406 Soil aggregate stability, soil physicochemical properties and root and litter traits were analysed by  
407 analysis of variance (ANOVA) and post hoc Tukey's honestly significant difference (HSD) test. The  
408 land use explanatory variable was treated as a factor. Relative abundance of the different phyla and  
409 trophic modes were analysed by ANOVA and post hoc HSD with land use and sample fraction as  
410 explanatory variables. All variables tested fulfilled ANOVA assumptions except for the Saprotroph  
411 trophic mode that was inverse transformed to fulfil ANOVA assumptions. The relationships between  
412 the relative abundance of bacterial phyla and the soil physicochemical variables, root and litter traits  
413 were tested using Spearman correlations.

414 Similarities/dissimilarities between microbial communities were displayed using non-metric multi-  
415 dimensional scaling (NMDS) of Bray–Curtis dissimilarity for bacterial and fungal species matrices.  
416 To indicate similarities between treatments on the NMDS configuration, the points on the NMDS  
417 ordination were overlapped with spider diagrams and convex hull polygons indicating land use and  
418 soil fraction was indicated with different symbols. Surfaces were added to show soil aggregate  
419 stability data on the ordinations. PERMANOVA tests (adonis R function) were performed for the  
420 bacteria and fungi species matrices as the response and the two different factors (land use and soil  
421 fraction) as the explanatory variables.

422 The Shannon diversity index for bacteria and fungi was calculated for each land use and sample  
423 fraction (rhizosphere and bulk soil samples). The relationships between the microbial community  
424 composition and the soil physicochemical variables, root and litter traits were tested using Spearman  
425 correlations between these variables and alpha (Shannon diversity index) and beta diversities (NMDS  
426 first and second axis) and a db-RDA. In order to select the environmental parameters to be included in  
427 the constrained ordination, an initial db-RDA including all parameters was performed followed by a  
428 stepwise model selection using Generalized Akaike Information Criterion (AIC, ordistep function  
429 with a backward direction). Finally, the db-RDA analysis was performed only for the variables  
430 obtained. ANOVA tests were performed on the final constrained ordination to confirm that the first  
431 two axes and the environmental variables and the final constrained ordination were significant.

#### 432 **2.4.2. Laboratory inoculation experiment**

433 First, we explored the differences on soil, vegetation and root traits and aggregate stability among the  
434 different combinations of treatments: i) origin (control, arable and woodland), ii) inoculation (not  
435 inoculated, arable and woodland) and iii) root treatment (no roots, fibrous and tap). Soil  
436 physicochemical properties, aboveground vegetation biomass, root traits, aggregate stability and  
437 aggregate fractions were analysed by ANOVA when ANOVA assumptions were fulfilled and with  
438 Kruskal Wallis tests when they were not fulfilled. Origin, inoculation, and root treatment were used as  
439 explanatory variables and were treated as factors. PCA was performed with a selection of variables

440 used for the description of soil, vegetation, and root properties. Variables included in the PCA were:  
441 aboveground biomass, CO<sub>2</sub> assimilation, soil water content, soil pH, SOC, soil C:N, SRL, VFR,  
442 MRD, RMD, RLD, root C:N, GRSP<sub>T</sub>, ergosterol and MWD. These variables were selected based on  
443 their correlation and ecological significance (i.e. variables that were correlated but their source was  
444 ecologically different were kept, e.g. soil C:N and root C:N). Apart from the ANOVA performed for  
445 MWD with soil origin, inoculation and root treatment as factors, an additional analysis of covariance  
446 (ANCOVA) model was tested and included RLD to explore separately the effect of root chemical (i.e.  
447 species effect) and root physical properties (quantity of roots) within the effect of root treatment.

448 Non-metric multi-dimensional scaling (NMDS) of Bray–Curtis dissimilarity were performed for  
449 bacterial and fungal species matrices obtained for bulk soil fraction (since the rhizosphere sample  
450 fraction was only available for treatments where roots were present). To indicate similarities between  
451 treatments on the NMDS configuration, the points on the NMDS ordination were overlapped with  
452 convex hull polygons indicating the inoculation treatment, origin was indicated with symbols of  
453 different colour and root treatment was indicated with different symbol shape. PERMANOVA tests  
454 (adonis R function) were performed for the bacteria and fungi species matrices as the response and the  
455 three different treatments (origin, inoculation and root treatment) as the explanatory variables using  
456 species matrix of bulk soil and rhizosphere fractions. Additional Analysis of similarities (ANOSIM)  
457 were performed to test the effects of root treatment, and inoculation in each of the combinations of the  
458 experimental design in bulk soil sample fractions.

459 The Shannon diversity index for bacteria and fungi was calculated for each pot. In line with the field  
460 study, Spearman correlations between soil, vegetation, root traits and aggregate stability variables and  
461 alpha (Shannon diversity index) and beta diversities (NMDS first and second axis) were calculated.  
462 Likewise, a db-RDA was performed on species matrices of bacterial and fungal communities obtained  
463 from the bulk soil fraction. Environmental parameters included in the db-RDA were selected by a  
464 stepwise model selection process using AIC similar to the process performed for the field study.  
465 ANOVA tests were performed on the final constrained ordination to confirm that the first two axes  
466 and the environmental variables and the final constrained ordination were significant.

467 Finally, we explored the textural class (proportion of clay, fine silt, coarse silt, fine sand, coarse sand)  
468 of soil aggregates used for the stability tests, to examine whether there was any possible influence of  
469 inoculation on the results of stability tests. We depicted a soil texture triangle to show the texture of  
470 the different treatment combinations and overlapped with ellipses discriminating the origin of the  
471 sample and symbols of different colours indicating inoculation treatment. Then, we performed a  
472 weighted Classical (Metric) Multidimensional Scaling with textural properties to explore the  
473 differences among origin and inoculation treatment, the points on this ordination were overlapped  
474 with convex hull polygons indicating the origin, and inoculation was indicated with symbols of  
475 different colour. Finally, we performed an analysis of similarities (ANOSIM) to test the effects of  
476 origin and inoculation treatments on the textural properties of aggregates.

477 All analyses were performed in RStudio Version 3.6.0 (R Core Team, 2019) using the vegan  
478 (Oksanen et al., 2019) and ade4 (Dray and Dufour, 2007) packages.

### 479 **3. Results**

#### 480 **3.1. Field study: Relationships between soil aggregate stability and microbial communities** 481 **among land use types**

##### 482 **3.1.1. Relationships between land use and soil, litter and root properties**

483 Soil physicochemical properties from samples taken at depths of 0-0.1 m and 0.2-0.3 m, differed  
484 between land uses (Table 1). The arable field site had a higher pH and nutrient contents (P, K) but  
485 lower Mg, SOC, N and C than the other sites. The pasture site had the highest SOC, N and C contents.  
486 All soils had a loamy texture, but ancient and secondary woodlands and arable field sites possessed a  
487 clay loam texture and both the grassland and pasture had a sandy loam texture. Roots had similar  
488 traits between ancient and secondary woodlands (Table S2), with lower SRL ( $19.68 \pm 4.06$  and  
489  $13.16 \pm 1.16$  m g<sup>-1</sup> respectively), lower MRD ( $0.44 \pm 0.01$  and  $0.56 \pm 0.02$  mm respectively), lower RLD  
490 ( $5.69 \pm 1.26$  and  $5.12 \pm 0.90$  km m<sup>-3</sup>), higher N content ( $1.18 \pm 0.06$  and  $1.48 \pm 0.01$  mg g<sup>-1</sup> respectively)  
491 and lower C:N ( $35.04 \pm 2.57$  and  $27.72 \pm 0.69$  respectively) than the other land use types. Litter traits  
492 had similar trends to that observed in roots: litter from ancient and secondary woodlands had higher  
493 hemicellulose ( $17.02 \pm 1.01$  and  $19.88 \pm 0.20$  mg g<sup>-1</sup> respectively) and N content ( $1.37 \pm 0.07$  and  
494  $1.43 \pm 0.02$  mg g<sup>-1</sup> respectively) and lower C:N ( $26.62 \pm 1.35$  and  $26.21 \pm 1.08$  respectively), compared to  
495 the other land use types. Soil aggregate stability was significantly different among land uses (Fig. 3),  
496 with pasture having the greatest MWD and arable land the lowest, with the other three land uses  
497 possessing an intermediate behaviour. A clear effect of soil depth was also identified (Fig. 3),  
498 highlighting the role of processes at the surface that promote aggregate stability and justifying the  
499 subsequent focus on soil sampled at a depth of 0-0.1 m.

##### 500 **3.1.2. Effects of land use on the structure of bacterial and fungal communities**

501 Land use type affected the relative abundance of Actinobacteria, Proteobacteria and Verrucomicrobia  
502 (Fig. 4a) with the arable land having a significantly higher relative abundance of Actinobacteria and  
503 lower relative abundance of Proteobacteria and Verrucomicrobia compared to other land uses. In  
504 addition, a significant interaction was found between land use and sample fraction for Acidobacteria,  
505 with a decreased relative abundance in woodland rhizosphere soils but not in woodland bulk soil

506 samples. Sample fraction also significantly affected the relative abundance of Bacteroidetes and  
507 Verrucomicrobia (Fig. 4a), which were more abundant in bulk soils for Bacteroidetes and the opposite  
508 for Verrucomicrobia, with only a significant effect of land use type in the rhizosphere for  
509 Verrucomicrobia. Correlations between relative abundance of bacterial phyla and soil, root and litter  
510 variables showed that oligotrophic Chloroflexi and Verrucomicrobia were significantly correlated to  
511 root and litter physicochemical variables (i.e. SRL, VFR, MRD, RDMC, root and litter N and C:N,  
512 litter lignin) in the rhizosphere but not in the bulk soil (Table 2). Conversely, copiotrophic phyla  
513 (Actinobacteria, Bacteroidetes and Proteobacteria) showed similar correlations in bulk soil but not in  
514 the rhizosphere. The relative abundance of these copiotrophic phyla were correlated with some soil  
515 physicochemical traits (pH, P, sand content) and for the specific case of Actinobacteria, this phylum  
516 showed significant correlations with root and litter traits (SRL, RMD, root N, C:N and hemicellulose  
517 and litter fibres, Table 2). For fungi, land use influenced the relative abundance of Ascomycota and  
518 Zygomycota, with a significant effect of sample fraction for Ascomycota (Fig. 4b). Arable land had  
519 the highest relative abundances of Ascomycota, whilst the secondary woodland possessed the lowest  
520 relative abundance of Ascomycota and the highest abundance of Zygomycota. Regarding trophic  
521 mode, land use type affected the relative abundance of saprotrophs and symbiotrophs with the arable  
522 field having the lowest abundances. The land use type also influenced the relative abundance of fungi  
523 belonging to an unspecific category that includes pathotrophs, saprotrophs and symbiotrophs, with the  
524 highest relative abundance in secondary woodland (Fig. 4c).

525 NMDS ordinations showed that bacterial and fungal communities were markedly distinct between  
526 land use types (Figure 5a). These findings were supported by the PERMANOVA performed for the  
527 bacterial and fungal species matrices (Table 3), which showed that land use type was by far the most  
528 important factor structuring bacterial and fungal communities ( $R^2=0.44$   $p=0.01$  and  $R^2=0.53$   $p=0.01$ ,  
529 respectively) irrespective of whether communities were taken from the rhizosphere or bulk soils  
530 (Table 3). Significant trends between changes in community structure and soil aggregate stability  
531 were also observed, with arable communities having the lowest MWD (Figure 5a).



532 **3.1.3. Relationships between environmental variables and bacterial and fungal communities**  
533 **among land use types**

534 The results of the relationships between soil physicochemical properties, root and litter traits (Tables 1  
535 and S2, Figure 3) and microbial community composition are shown in the db-RDA (Figure 5b) and  
536 Spearman correlations with alpha and beta diversities (Table S4). The final db-RDA analysis (Figure  
537 5b) shows only the environmental parameters that were selected through stepwise model selection:  
538 MWD, soil pH and P, and MRD for bacterial communities and MWD, RLD, FR and soil pH and P for  
539 fungal communities. There was a clear separation between land uses for bacterial communities  
540 (Figure 5b), with three main groups: i) woodlands (ancient and secondary), ii) grasslands (actively  
541 and not actively grazed) and iii) arable land. Soil from woodlands had significantly greater MWD and  
542 higher MRD, whereas arable land had a significantly greater soil pH and P (Figure 5b). For fungal  
543 communities, a similar configuration was found among the three land use types, but soil from  
544 grasslands had a significantly higher RLD, and woodlands had a greater FR. ANCOVA tests  
545 performed on the final constrained ordination confirmed that the first two axes, the environmental  
546 variables and the final constrained ordination were significant.

547 Bacterial and fungal alpha diversities (Table S3) were not significantly correlated with any of the  
548 variables measured (Table S4). Bacterial beta diversity was significantly and positively correlated  
549 with soil pH, clay and magnesium contents, soil C:N, FR, MRD, root and litter N and litter lignin and  
550 negatively correlated to MWD, sand content, SRL, VFR and root and litter C:N and hemicellulose.  
551 Similarly, fungal beta diversity was significantly and positively correlated ( $p < 0.001$  and  $R^2 \geq 0.65$ )  
552 with soil pH, clay and magnesium contents, soil C:N, FR, MRD, root N, and litter lignin, and  
553 negatively with sand content, SRL, VFR, RLD, root C:N and root and litter hemicellulose (Table S4).

554 **3.2. Laboratory inoculation experiment: Effect of microbial inoculation on soil aggregate**  
555 **stability and interactions with soil origin and root systems.**

556 **3.2.1. Effects of soil origin, inoculation and root systems on soil properties and plant traits**

557 The PCA performed with a selection of variables used for the description of soil, plant and root traits  
558 explained 56.7% of the variance (Fig. 6). The first PCA axis (horizontal), accounted for 43.7% of the  
559 variation and the second PCA axis accounted for 13% of the variation. This PCA showed a clear  
560 differentiation between control, and either arable land or woodland. The main contributors to these  
561 differences were soil pH, SOC and MWD. Control soil had significantly higher pH, soil and root C:N  
562 ratios and lower SOC, MWD, GRSP<sub>T</sub> and ergosterol content compared to soils from the arable land  
563 and woodland.

564 All soil properties assessed (e.g. pH, SOC, C, N) were influenced significantly by the origin of the  
565 soil (i.e. control, arable and woodland, Table S5,  $p < 0.05$ ). Additionally, a significant effect of  
566 inoculation was found for soil water content ( $p < 0.001$ ), which may be because this was the only  
567 variable that was measured in the top 0.1 m of the soil profile, therefore including the soil used for  
568 inoculation. Aboveground plant biomass was significantly affected by soil origin and root system type  
569 ( $p < 0.001$ ) but not by inoculation. Concerning root traits, soil origin affected significantly root biomass  
570 ( $p = 0.014$ ), SRL, VFR, FR, MRD, N and C:N ( $p < 0.001$ ) and root system type affected root biomass  
571 ( $p < 0.001$ ), SRL ( $p < 0.020$ ), RMD and RLD ( $p < 0.001$ ) but no effect of inoculation was found (Table  
572 S5, Fig. 7).

### 573 **3.2.2. Effects of soil origin, inoculation and root systems on the structure of bacterial and fungal** 574 **communities.**

575 NMDS ordinations showed that bacterial and fungal communities were markedly distinct between  
576 inoculation treatments and soil origin but there were weaker effects of root system types (Figure 8a).  
577 These findings were supported by the PERMANOVA performed for the bacterial and fungal species  
578 matrices (Table 4), which showed that the main factors structuring bacterial communities were  
579 inoculation ( $R^2 = 0.18$ ,  $p < 0.001$ ) and origin ( $R^2 = 0.05$ ,  $p < 0.001$ ) in bulk soil, and inoculation ( $R^2 = 0.26$ ,  
580  $p < 0.001$ ), origin ( $R^2 = 0.09$ ,  $p < 0.001$ ) and root system ( $R^2 = 0.06$ ,  $p < 0.001$ ) in the rhizosphere. The  
581 results observed for fungal communities were different, with inoculation ( $R^2 = 0.16$ ,  $p < 0.001$ ), origin  
582 ( $R^2 = 0.03$ ,  $p < 0.001$ ) and root system ( $R^2 = 0.02$ ,  $p < 0.001$ ) significantly affecting fungal communities in

583 the bulk soil. However, for fungal communities in the rhizosphere, only inoculation treatments  
584 ( $R^2=0.24$ ,  $p<0.001$ ) and soil origin ( $R^2=0.08$ ,  $p<0.001$ ) had significant effects. Overall, considering  
585 the  $R^2$  values, the influence of root system type was stronger on the structure of bacterial communities  
586 than for fungal communities. Furthermore, the interaction between origin and inoculation was  
587 significant in all cases ( $p<0.001$ ), indicating that the effect of inoculation was affected by the soil  
588 origin. Additionally, for fungal communities in the bulk soil, the effect of root system type was  
589 affected by both inoculation (inoculation\*root treatment  $R^2=0.03$ ,  $p<0.001$ ) and soil origin  
590 (origin\*root treatment  $R^2=0.03$ ,  $p=0.042$ ). The overall plant root system effects were minor compared  
591 to soil properties, nevertheless significant effects of root systems in certain soils were observed (Table  
592 5). The effect of root system type was context-specific: in the non-inoculated control soil both  
593 bacterial and fungal communities were significantly affected ( $p=0.013$  and  $0.012$  respectively), while  
594 for the field-sampled soils root system significantly influenced fungal communities only in arable soil  
595 non-inoculated ( $p=0.001$ ) and inoculated with woodland soil ( $p=0.015$ ). In contrast, inoculation  
596 affected both bacterial and fungal communities ( $p<0.05$ ) for all combinations of treatments.

597 The dbRDA constrained ordinations of bacteria and fungi species matrices showed that the main  
598 factors affecting the structure of microbial communities were MWD, RMD and soil C:N for bacterial  
599 communities and MWD, soil C, RMD, GRSP<sub>T</sub> and ergosterol for fungal communities (Fig. 8b).

600 ANCOVA tests performed on the final constrained ordination confirmed that the first two axes, the  
601 environmental variables and the final constrained ordination were significant. Bacterial and fungal  
602 alpha diversities did not show significant correlations with any of the variables measured (Table S6).

603 Bacterial beta diversity was positively correlated ( $p < 0.001$  and  $R^2 \geq 0.3$ ) with some soil properties  
604 (SOC, N, C, GRSP<sub>EE</sub> and MWD) and with root N, and negatively correlated with soil pH, soil C:N,  
605 CaCO<sub>3</sub> and root C:N. Fungal beta diversity was significantly and negatively correlated ( $p < 0.001$  and  
606  $R^2 \geq 0.3$ ) with soil pH and CaCO<sub>3</sub>, but positively correlated with MWD and ergosterol (Table S6). It  
607 is interesting to note that GRSP was strongly related to bacterial beta diversity, even more than with  
608 fungal beta diversity, lending support to the increasing evidence that GSRP is not solely of AFM  
609 fungal origin (Cissé et al., 2020).

610 **3.2.3. Effects of soil origin, inoculation and root treatments on soil aggregate stability.**

611 Soil aggregate stability (MWD) was significantly affected by soil origin ( $p < 0.001$ ) and inoculation  
612 ( $p = 0.04$ ) but not by root system type (Table 6, Figure 7a). The inoculation increased aggregate  
613 stability in the poorly structured control soil, but not in arable and woodland soils (Figure 7a). The  
614 effect of inoculation was mainly on macroaggregates and their dispersion (fractions 1-2 mm, 0.5-1  
615 mm and 0.1-0.2 mm; Table S5). The ANCOVA model including RLD showed that the effect of root  
616 system type was again not significant, but a significant interaction did occur between inoculation and  
617 RLD, revealing that the number of roots modified the effect of inoculation ( $p < 0.001$ ; Table 6). The  
618 correlations between aggregate stability and the reputed fungal markers  $GRSP_T$ ,  $GRSP_{EE}$  and  
619 ergosterol were only significant when all three soils were analysed together (Fig. S4), in agreement  
620 with the results of ANOVA tests that showed that only the effect of soil origin was significant (Table  
621 S5). The analysis of the textural properties of soil aggregates used for the aggregate stability tests  
622 showed that removing the top 15 mm after the experiment for all soil analyses was efficient and there  
623 was no possible influence of soil addition on the results of stability tests (Fig. S5a and S5b), since the  
624 texture of aggregates used for the tests was related to origin ( $p = 0.001$ ) but not to inoculation  
625 ( $p = 0.015$ , Fig. S5c).

626

#### 627 **4. Discussion**

628 *Hypothesis 1: There are strong relationships between soil aggregate stability and microbial*  
629 *community structure that are driven by land use.*

630 In agreement with our first hypothesis, the impact of land use significantly affected soil aggregate  
631 stability and the structure of microbial communities. A large body of previous research has found that  
632 the conversion of natural forest to cropland results in a decline of soil quality, SOC and aggregate  
633 stability and the opposite is true for soils restored after a disturbance (Barto et al., 2010; Duchicela et  
634 al., 2013; Delelegn et al., 2017). Positive relationships between SOC and aggregate stability have  
635 been found in different soil types, such as black soils (Zhang et al., 2012), Typic Ustochrepts (Saha et  
636 al., 2011), and loamy soils (Vermic Haplubrepts) where SOC is thought to increase aggregate stability  
637 by lowering the wettability and increasing the cohesion of aggregates (Chenu et al., 2000). Thus,  
638 differences in SOC might explain the lowest aggregate stability in the arable field and the highest  
639 stability in pasture and grassland. Apart from this influence of SOC on MWD, we found other factors  
640 influencing MWD in our land use gradient that impact aggregate stability, as described previously: i)  
641 agricultural practices such as the mechanical action of tillage (Balesdent et al., 2000), management  
642 inputs (e.g. fertilizer, herbicide (Steenwerth et al., 2002)) and grazing (Barto et al., 2010), ii) the  
643 presence of roots (measured through RLD; Hudek et al. (2017)), iii) the higher C:N in roots and litter  
644 implying a higher content in recalcitrant matter that influences fungal activity and MWD as a  
645 consequence (Bossuyt et al., 2001).

646 We demonstrated a strong impact of land use gradient on the structure of bacterial and fungal  
647 communities. A regional study in Great Basin Province (California, USA) identified that agricultural  
648 management can have larger effects on soil microbial communities than elevation and precipitation  
649 gradients (Drenovsky et al., 2010). However, fungal and bacterial communities differ in their  
650 responses to changes in agricultural practices. Previous research has shown that tillage reduces  
651 microbial biomass and abundance of AMF (Helgason et al., 2010; Zhang et al., 2013) and that the  
652 structure of the microbial communities responds strongly to land use with higher proportions of fungi

653 observed in natural ecosystems compared with agro-systems (Fanin and Bertrand, 2016). In a study  
654 across a range of European field sites, land use intensification effects on microbial communities were  
655 stronger when land use change affected soil conditions such as pH, carbon, nitrogen and phosphorous  
656 contents (Thomson et al., 2015). Here, although we found significant relationships between the  
657 changes in microbial community structure, aggregate stability and soil properties along the land use  
658 gradient, causality cannot be confirmed.

659 *Hypothesis 2: The arable field site with lower aggregate stability will have a lower relative*  
660 *abundance of copiotrophic communities and reduced relative abundances of saprotrophic and*  
661 *ectomycorrhizal fungi, but the rhizosphere niche will favour copiotrophic bacterial communities.*

662 Given that microaggregates are considered to hold oligotrophic communities and macroaggregates  
663 favour copiotrophic communities due to their differences in contents of labile/recalcitrant carbon  
664 (Trivedi et al., 2017), we expected to find an increase in the relative abundance of copiotrophic  
665 communities in soils with higher soil aggregate stability (i.e. higher MWD after disruption). In line  
666 with this hypothesis, the arable field (with the lowest soil aggregate stability), had a lower relative  
667 abundance of copiotrophic Proteobacteria phyla. However, we did not observe similar trends for  
668 copiotrophic Actinobacteria and Bacteroidetes, and oligotrophic Acidobacteria, Chloroflexi and  
669 Verrucomicrobia. In addition, correlations between relative abundance of bacterial phyla and MWD  
670 were not significant. However, other soil physicochemical factors and notably root and litter  
671 physicochemical traits, affected the relative abundance of these phyla with a differential effect on bulk  
672 and rhizosphere niches. Lauber et al. (2008) also showed that rather than land use itself, changes in  
673 soil edaphic properties explained changes in life strategies of bacterial communities. Since  
674 Chloroflexi and Verrucomicrobia were correlated to root and litter physicochemical variables in the  
675 rhizosphere but not in the bulk soil, we conclude that oligotrophic bacteria are more dependent on  
676 rhizosphere conditions where labile carbon originates mostly from rhizodeposits, exudates and  
677 mucigel (Dennis et al., 2010). On the contrary, copiotrophic phyla were more dependent on bulk soil  
678 conditions where there are more exopolysaccharides released into the soil by the microorganisms  
679 themselves. The relative abundance of these copiotrophic phyla was correlated with some soil

680 physicochemical traits (pH, P, sand content) and for the specific case of Actinobacteria, this phylum  
681 showed correlations with root and litter traits (SRL, RMD, root N, C:N and hemicellulose and litter  
682 fibres, Table 2). This particular behaviour of Actinobacteria could be because Actinobacteria exhibits  
683 both copiotrophic and oligotrophic life strategies consistent with their capacity to degrade both labile  
684 and complex carbon substrates (Ho et al., 2017). Bergmann et al. (2011) found that Verrucomicrobia  
685 are oligotrophic with a highly variable relative abundance across soils, and that members of this  
686 phylum were most abundant in soils from grasslands and prairies. However, these authors  
687 acknowledged that the ecology of Verrucomicrobia remains poorly understood, and different factors  
688 may affect the distribution of this phylum.

689 Studies of bacterial communities inhabiting the rhizosphere in grasslands (Mao et al., 2014; Shi et al.,  
690 2015) and croplands (Peiffer et al., 2013; Donn et al., 2015) found that rhizosphere samples are  
691 enriched in Proteobacteria, Actinobacteria, and Bacteroidetes. However, here we found an enrichment  
692 of Verrucomicrobia (oligotrophic) and diminution of Bacteroidetes (copiotrophic) and Acidobacteria  
693 (oligotrophic) in rhizosphere communities. Thus, the hypothesis that the rhizospheric niche favours  
694 copiotrophic bacterial taxa (Peiffer et al., 2013; Lladó and Baldrian, 2017) was only confirmed with  
695 the reduced relative abundance of the oligotrophic Acidobacteria, highlighting the heterogeneous  
696 nature of the rhizosphere and the importance of studying the relationships between soil, root and  
697 physicochemical variables and the relative abundance of trophic modes in both rhizosphere and bulk  
698 soils that are detailed above.

699 Fungal symbiotrophs and saprotrophs are among the most sensitive to management practices  
700 (Hartmann et al., 2012; Orgiazzi et al., 2012; Mueller et al., 2016). For example, Orgiazzi et al. (2012)  
701 found that ectomycorrhizal phylotypes were numerous in natural sites with trees but were missing in  
702 anthropogenic and grass-covered sites, whereas Lauber et al. (2008) found that coprophilous fungi  
703 were common in grazed sites. Management disturbances have been found to increase the relative  
704 abundance of Ascomycota and decrease Basidiomycota (Thomson et al., 2015). Similarly, we  
705 observed the expected decrease in fungal saprotrophs and symbiotrophs at the arable site and a higher

706 relative abundance of Ascomycota, although a large proportion of unidentified trophic modes may be  
707 influencing our results.

708 *Hypothesis 3: Microbial inoculation of soils and dense root systems increase soil aggregate stability*  
709 *in unstructured soils.*

710 Inoculation of live soil communities had large impacts on the sterilised soil communities, irrespective  
711 of soil origin (sterilised control, arable or woodland). However, the effect of the inoculation on the  
712 structure of bacterial communities was dependent on the origin of the soils. This result agrees with  
713 previous studies that found that texture and pore size distribution strongly determined the fate of  
714 introduced microorganisms (Rutherford and Juma, 1992; Huysman and Verstraete, 1993; van Veen et  
715 al., 1997). Additionally, the effect of root system type was only evident on bacterial communities in  
716 the rhizosphere, but not on communities inhabiting bulk soil, which is likely due to differences in root  
717 length and the increased microbial numbers in the rhizosphere carbon rich habitat (Jones et al., 2009).  
718 In a previous study, we also found that the interactions between microbial community structure and  
719 root traits were more intense in communities isolated from rhizosphere compared to bulk soil, and that  
720 bacterial community composition was better explained by root traits than in fungal communities  
721 (Merino-Martín et al., 2020). We showed that root system type only affected the structure of bacterial  
722 communities in control soils that were not inoculated, stressing that the role of roots was secondary  
723 compared to inoculation and initial soil structure. Correspondingly, root system type affected fungal  
724 community structure in non-inoculated control soil, but it also affected significantly the non-  
725 inoculated arable soil and arable soil with woodland inoculum, which may be because it had the  
726 lowest relative abundance of fungal communities and the least developed mycorrhizal networks.  
727 These limited effects of root system type are in agreement with other studies that proposed a hierarchy  
728 in the contribution of soils and root systems (traits and exudates), with soil physicochemical  
729 properties contributing most strongly to the microbiome and root traits and exudates gradually altering  
730 this soil microbiome (Bever et al., 2012; Philippot et al., 2013; van der Putten et al., 2013).

731 As hypothesized, the microbial inoculation with soils increased aggregate stability in the poorly  
732 structured control soil, but not in arable (with low aggregate stability) and woodland soils (with high



733 aggregate stability). This finding is in line with Barto et al. (2010), who concluded that, in highly  
734 aggregated soils, abiotic factors can be more important for determining soil aggregation than biotic  
735 factors. However, besides the lack of initial structure, the control soil also showed very distinct  
736 physicochemical properties that can influence the effect of microbial inoculation on aggregate  
737 stability, such as lower contents of SOC and soil N, and higher pH and CaCO<sub>3</sub> content (Al-Ani and  
738 Dudas, 1988; Chenu et al., 2000; Wu et al., 2017). Even though the focus of our research was on  
739 bacterial and fungal communities, we acknowledge that our soil inoculation method may have  
740 included soil fauna, such as nematodes, protozoa, or collembolans that were not explored in our study  
741 (van de Voorde et al., 2012).

742 *Hypothesis 4: A greater effect of inoculation on aggregate stability is expected in soils inoculated*  
743 *with woodland soil.*

744 Surprisingly, we did not observe greater aggregate stability in soil inoculated with woodland  
745 compared to arable soil. These two soils, woodland and arable, were intrinsically similar but with  
746 significantly different aggregate stability. In a similar experiment, Duchicela et al. (2012) found  
747 greater stable aggregates in pots inoculated with soil communities from remnant grasslands, compared  
748 to soils inoculated with communities from disturbed post-agricultural sites. These authors suggested  
749 that the use of the same suite of native prairie plant species across both remnant and disturbed soils in  
750 their experiment could explain this effect, reflecting local coadaptation (Duchicela et al., 2012).  
751 Furthermore, the effects of inoculation may also depend on the starting point, the incubation time and  
752 conditions after inoculation and not only on the inoculum used.

753 Several laboratory inoculation experiments also revealed that mycorrhizal fungi play an important  
754 role in stabilizing aggregates. Harris et al. (1964) found that even if bacteria were more important than  
755 fungi in the primary stabilization of microaggregates, fungi were involved in the stabilization of  
756 macroaggregates. Bearden and Petersen (2000) found that AM fungi contributed to the stabilization of  
757 aggregates in a vertisol, and that the effect was significant after only one growing season. Bossuyt et  
758 al. (2001) found that macroaggregate formation was positively influenced by fungal activity but was  
759 not significantly influenced by residue quality or bacterial activity. Most of these inoculation studies

760 found that the mechanism by which mycorrhizal fungi increase the stability of aggregates is not only  
761 related to hyphae formation, but is also through the stimulation of root growth (Bearden and Petersen,  
762 2000; Graf and Frei, 2013; Bast et al., 2016). In agreement with these authors, we found that in the  
763 model including root length density (RLD), there was an effect of the interaction between the  
764 inoculation treatment and RLD on aggregate stability, which was remarkable, given the absence of the  
765 effect of root type.

766 We did not observe any effect of inoculation and root system type on the content of glomalin related  
767 soil proteins (GRSP) or ergosterol. A significant correlation between these compounds and aggregate  
768 stability was found, but it was driven by the different origins of the soils. The legacy effect of soil  
769 origin on these compounds emphasizes their recalcitrant nature that has been widely discussed in  
770 previous research (Lorenz et al., 2007; Gispert et al., 2013a; Cissé et al., 2020). In addition, GRSP  
771 does not appear to be a good predictor of fungal activity, nor of increased physical stability.

## 772 **6. Conclusions**

773 Our results, from field and experimental approaches, depict the complex relationships between soil  
774 properties, land use and plant traits shaping microbial communities and affecting soil structure. Our  
775 field study demonstrated the existence of strong relationships between land use, microbial  
776 communities and soil aggregate stability. Even though we did not find consistent relationships  
777 between land use gradient, aggregate stability and the relative abundance of specific bacterial trophic  
778 modes, the importance of root traits shaping the relative abundance of bacterial phyla was  
779 demonstrated. Rhizosphere conditions shaped bacterial communities with oligotrophic bacteria  
780 conditioned by the rhizospheric environment, where labile C is found, whilst copiotrophic phyla were  
781 more dependent on bulk soil conditions. Fungal communities responded to the land use gradient with  
782 a lower relative abundance of saprophytic and symbiotic in disturbed sites. The inoculation  
783 experiment showed that microbial inoculation affects aggregate stability in the control soil (that had  
784 very low aggregate stability initially), but not in soils sterilized from the arable field and the  
785 secondary woodland, possibly because in aggregated soils, abiotic factors can be more important for

786 determining soil aggregation than biotic factors. We did not observe differential effects of the  
787 different inoculums (arable *versus* secondary woodland), most likely due to the short period of the  
788 inoculation experiment. Again, the importance of roots for bacterial communities in the rhizosphere  
789 niche was highlighted in the inoculation experiment, with root system type affecting only the structure  
790 of bacterial communities but not fungal communities, and only in the rhizosphere but not in bulk  
791 soils. Additionally, root system type only affected the structure of bacterial communities in control  
792 soils that were not inoculated, stressing that the role of roots was secondary compared to inoculation  
793 and initial soil structure. Finally, even though we did not find a significant effect of root system type  
794 on aggregate stability, we have shown that the effect of the microbial inoculation was modulated by  
795 root length density, highlighting the role of roots in shaping microbial communities and their effects  
796 on soil structure.

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804 lives to overcome the pandemic.

805 **Tables:**

806 **Table 1.** Soil physicochemical properties of sites selected for the field study (mean of the six samples  
 807 collected at depths of 0-0.1 m and 0.2-0.3 m). Sand, silt, clay, soil organic carbon (SOC), total  
 808 nitrogen (N) and total carbon (C) are expressed in % w/w. Available P, K and Mg are expressed in  
 809 mg/l. Carbon:nitrogen (C:N) ratio is expressed in :1. F and p values are shown. Post hoc comparisons

	<b>Ancient Woodland</b>	<b>Secondary Woodland</b>	<b>Grassland</b>	<b>Pasture</b>	<b>Arable</b>	<b>F value</b>	<b>Pr(&gt;F)</b>
<b>pH</b>	5.87 ± 0.16 a	6.38 ± 0.15 ab	6.30 ± 0.15 a	6.10 ± 0.06 a	7.00 ± 0.22 b	7.376	<0.001
<b>P</b>	12.73 ± 2.44 a	10.50 ± 2.53 a	14.10 ± 2.03 a	20.07 ± 2.38 a	80.47 ± 2.47 b	157.2	<0.001
<b>K</b>	321.17 ± 28.01 a	253.50 ± 41.88 a	254.67 ± 29.70 a	205.67 ± 15.24 a	595.00 ± 49.26 b	19.99	<0.001
<b>Mg</b>	256.33 ± 10.19 b	247.50 ± 5.91 ab	229.50 ± 7.10 ab	222.50 ± 5.46 ab	209.00 ± 16.65 a	3.661	0.018
<b>Sand</b>	35.00 ± 2.85 ac	25.67 ± 2.63 a	40.17 ± 5.31 bc	52.67 ± 2.76 b	29.17 ± 1.47 ac	10.57	<0.001
<b>Silt</b>	41.50 ± 1.15 a	44.00 ± 0.52 a	38.33 ± 2.62 ab	32.83 ± 1.58 b	40.50 ± 1.02 a	7.416	<0.001
<b>Clay</b>	23.50 ± 1.88 ab	30.33 ± 2.28 a	21.50 ± 2.92 ab	14.50 ± 1.23 b	30.33 ± 2.23 a	9.331	<0.001
<b>Texture</b>	Clay Loam	Clay Loam	Sandy Loam	Sandy Loam	Clay Loam	-	-
<b>SOC</b>	11.28 ± 1.34 bc	13.60 ± 1.38 abc	14.78 ± 1.37 ab	17.43 ± 1.46 a	8.52 ± 0.29 c	7.435	<0.001
<b>Total Nitrogen</b>	0.46 ± 0.06 ab	0.59 ± 0.05 ab	0.66 ± 0.05 ac	0.87 ± 0.06 c	0.42 ± 0.01 b	12.76	<0.001
<b>Total Carbon</b>	5.25 ± 0.73 b	6.52 ± 0.74 ab	6.97 ± 0.68 ab	8.56 ± 0.75 a	4.36 ± 0.12 b	6.202	<0.001
<b>C:N</b>	11.18 ± 0.25 a	11.02 ± 0.40 a	10.42 ± 0.30 a	9.85 ± 0.18 a	10.52 ± 0.46 a	2.51	0.067

810 were performed with Tukey HSD.

811

812 **Table 2.** Spearman's correlation coefficients between relative abundance of the different phyla of  
 813 Bacteria and soil properties, root and litter traits for bulk soil and rhizosphere sample fractions in the  
 814 field study (\*\*\*\*P < 0.0001, \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05). Correlations  $\geq 0.65$  are  
 815 highlighted in bold and correlations present in only one sample fraction are highlighted in red.

	Bulk soil						Rhizosphere					
	Oligotrophic			Copiotrophic			Oligotrophic			Copiotrophic		
	Acidobacteria	Chloroflexi	Verrucomicrobia	Actinobacteria	Bacteroidetes	Proteobacteria	Acidobacteria	Chloroflexi	Verrucomicrobia	Actinobacteria	Bacteroidetes	Proteobacteria
<b>Soil</b>												
MWD	0.01	-0.26	-0.06	-0.13	0.38	0.38	0.41	0.13	-0.18	-0.53	0.38	0.2
pH	-0.3	<b>0.72**</b>	-0.08	0.44	<b>-0.86**</b>	<b>-0.84****</b>	0.2	0.4	-0.08	0.32	0.07	<b>-0.87****</b>
P	-0.08	0.39	-0.48	<b>0.67**</b>	-0.29	-0.44	0.45	0.51	-0.64*	0.2	-0.12	-0.07
Potassium	-0.2	0.49	-0.11	0.390	-0.4	-0.460	-0.09	0.2	-0.06	0.370	-0.35	-0.130
Magnesium	-0.07	-0.38	0.45	<b>-0.71**</b>	0.24	0.38	<b>-0.69**</b>	-0.65*	0.67**	-0.5	0.23	0.38
Sand	0.24	-0.55*	-0.17	-0.12	<b>0.71**</b>	<b>0.70**</b>	0.01	-0.1	-0.11	-0.13	-0.02	0.60*
Silt	-0.39	0.25	0.46	-0.18	-0.61*	-0.43	-0.37	-0.17	0.49	0.04	0.18	-0.46
Clay	-0.09	0.48	0.1	0.04	-0.61*	-0.59*	-0.02	0.04	0.09	0.08	-0.03	-0.52
SOC	0.25	-0.29	-0.07	-0.25	0.47	0.5	0.14	-0.09	-0.02	-0.36	0.15	0.28
N	0.19	-0.17	-0.08	-0.21	0.39	0.4	0.18	-0.03	-0.02	-0.38	0.16	0.21
C	0.3	-0.24	-0.09	-0.25	0.44	0.46	0.14	-0.09	-0.01	-0.36	0.09	0.27
C:N	0.60*	-0.35	0.29	<b>-0.70**</b>	0.24	0.4	<b>-0.52</b>	<b>-0.73**</b>	0.59*	-0.04	-0.4	0.16
<b>Roots</b>												
SRL	-0.24	0.55*	-0.52*	<b>0.82***</b>	-0.18	-0.44	0.43	<b>0.80**</b>	<b>-0.69**</b>	0.47	-0.02	-0.21
VFR	-0.16	0.29	-0.55*	0.54*	0.17	-0.04	0.41	<b>0.75**</b>	<b>-0.68**</b>	0.19	0.15	0.06
FR	0.02	-0.24	0.52*	-0.38	-0.25	-0.08	-0.32	-0.64*	0.64*	-0.15	-0.1	-0.16
MRD	0.2	-0.47	0.56*	<b>-0.73**</b>	0.05	0.28	-0.44	<b>-0.80**</b>	<b>0.72**</b>	-0.42	-0.11	0.14
RDMC	-0.14	-0.60*	0.64*	-0.39	0.16	0.18	-0.42	<b>-0.90**</b>	<b>0.69**</b>	-0.34	-0.15	0.17
RMD	0	-0.35	-0.05	-0.18	0.35	0.52*	0.31	0.14	-0.16	-0.31	0.45	0.22
RLD	-0.07	-0.1	-0.3	-0.03	0.42	0.54*	0.16	0.36	-0.27	-0.2	0.42	0.43
N	0.21	0.08	0.56	<b>-0.77**</b>	-0.38	-0.13	-0.44	-0.59*	<b>0.71**</b>	0.01	-0.19	-0.32
C	0.31	<b>-0.72**</b>	0.04	-0.13	0.46	0.34	0.06	-0.42	-0.05	0.01	-0.09	0.09
C:N	-0.21	-0.08	-0.56	<b>0.77**</b>	0.38	0.13	0.44	0.59*	<b>-0.71**</b>	-0.01	0.19	0.32
lignin	-0.11	0.17	0.35	-0.35	-0.48	-0.32	-0.22	-0.15	0.45	0.22	0.22	-0.65*
celulose	0.06	-0.09	0.24	-0.02	-0.09	-0.26	0.26	-0.24	0.13	-0.3	-0.28	-0.19
hemicelulose	-0.11	0.04	-0.63*	<b>0.76**</b>	0.3	0.13	0.56	0.63*	<b>-0.78**</b>	-0.17	0.15	0.32
<b>Litter</b>												
N	0.11	-0.06	0.5	-0.54*	-0.19	0	-0.57*	-0.53	<b>0.68**</b>	0.13	-0.13	-0.21
C	-0.01	-0.03	-0.36	0.44	0.01	-0.12	0.60*	0.31	-0.45	-0.21	0.14	-0.19
C:N	-0.09	0.05	-0.54*	0.56*	0.17	-0.01	0.61*	0.51	<b>-0.68**</b>	-0.16	0.15	0.09
lignin	0.14	-0.29	0.61*	<b>-0.74**</b>	-0.1	0.24	-0.57	<b>-0.79**</b>	<b>0.76**</b>	0.27	-0.39	0.15
celulose	0.2	0	0.36	<b>-0.65*</b>	0.1	-0.02	-0.2	-0.28	0.35	-0.47	0.09	0.02
hemicelulose	-0.17	0.15	-0.47	<b>0.75**</b>	-0.01	-0.33	0.66*	0.68*	<b>-0.71*</b>	-0.17	0.35	-0.32

816  
 817 **Table 3.** Effects of land use and sample fraction and their interactions on the structure of bacterial and  
 818 fungal communities assessed with PERMANOVA. The degrees of freedom (Df), sum of squares (sum  
 819 of sqs), mean of squares (mean of sqs), the F. statistic, the proportion of the variance explained by  
 820 each model (R<sup>2</sup>) and probability (P) are shown.

Factors	Bacteria						Fungi					
	Df	Sums of sqs	Mean of sqs	F.Model	R <sup>2</sup>	Pr(>F)	Df	Sums of sqs	Mean of sqs	F.Model	R <sup>2</sup>	Pr(>F)
Land use	4	1.5469	0.38673	4.4095	0.438	0.001	4	4.8003	1.20008	6.3216	0.533	0.001
Samplefraction	1	0.1366	0.1366	1.5575	0.039	0.093	1	0.1365	0.13649	0.719	0.015	0.745
Land use*Samplefraction	4	0.1793	0.04483	0.5112	0.051	1	4	0.4629	0.11572	0.6096	0.051	0.993
Residuals	19	1.6663	0.0877		0.472		19	3.6069	0.18984		0.400	
Total	28	3.5292			1		28	9.0067			1	

821 **Table 4.** Effects of inoculation, origin and root treatments and their interactions on the structure of  
822 bacterial and fungal communities assessed with PERMANOVA. The degrees of freedom (Df), sum of  
823 squares (sum of sqs), mean of squares (mean of sqs), the F. statistic, the proportion of the variance  
824 explained by each model (R<sup>2</sup>) and probability (P) are shown.

Bulk soil												
Factors	Bacteria						Fungi					
	Df	Sums of sqs	Mean of sqs	F.Model	R <sup>2</sup>	Pr(>F)	Df	Sums of sqs	Mean of sqs	F.Model	R <sup>2</sup>	Pr(>F)
Inoculation	2	9.214	4.6072	18.5425	0.182	<b>0.001</b>	2	10.144	5.0721	15.0325	0.164	<b>0.001</b>
Origin	2	2.603	1.3017	5.239	0.051	<b>0.001</b>	2	1.703	0.8513	2.523	0.028	<b>0.001</b>
Rootsystem	2	0.709	0.3547	1.4277	0.014	0.074	2	1.109	0.5545	1.6434	0.018	<b>0.004</b>
Inoculation*Origin	4	2.185	0.5463	2.1986	0.043	<b>0.001</b>	4	2.622	0.6554	1.9424	0.042	<b>0.001</b>
Inoculation*Rootsystem	4	1.042	0.2604	1.0481	0.02	0.34	4	1.902	0.4756	1.4095	0.03078	<b>0.01</b>
Origin*Rootsystem	4	0.919	0.2297	0.9247	0.018	0.623	4	1.697	0.4243	1.2575	0.027	<b>0.042</b>
Inoculation*Origin*Rootsystem	8	1.745	0.2181	0.8777	0.034	0.818	t 8	3.142	0.3927	1.164	0.051	0.059
Residuals	130	32.301	0.2485		0.64		117	39.477	0.3374		0.63883	
Total	156	50.718			1.000		143	61.795			1.000	

Rhizosphere												
Factors	Bacteria						Fungi					
	Df	Sums of sqs	Mean of sqs	F.Model	R <sup>2</sup>	Pr(>F)	Df	Sums of sqs	Mean of sqs	F.Model	R <sup>2</sup>	Pr(>F)
Inoculation	2	3.5166	1.7583	12.2209	0.264	<b>0.001</b>	2	4.7153	2.35765	8.9631	0.241	<b>0.001</b>
Origin	2	1.1993	0.59963	4.1677	0.090	<b>0.001</b>	2	1.5272	0.7636	2.903	0.078	<b>0.001</b>
Rootsystem	1	0.8523	0.85235	5.9242	0.064	<b>0.001</b>	1	0.3279	0.32786	1.2464	0.017	0.182
Inoculation*Origin	4	1.4709	0.36771	2.5558	0.110	<b>0.001</b>	4	2.0624	0.51559	1.9601	0.105	<b>0.001</b>
Inoculation*Rootsystem	2	0.394	0.197	1.3692	0.03	0.086	2	0.6301	0.31504	1.1977	0.03218	0.167
Origin*Rootsystem	2	0.3101	0.15505	1.0777	0.023	0.329	2	0.5685	0.28424	1.0806	0.029	0.312
Inoculation*Origin*Rootsystem	4	0.7098	0.17746	1.2334	0.053	0.094	4	1.066	0.2665	1.0132	0.054	0.429
Residuals	34	4.8918	0.14388		0.37		33	8.6803	0.26304		0.44338	
Total	51	13.3448			1.000		50	19.5776			1.000	

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827 **Table 5.** Effects of root system type and inoculation in each of the combinations of the experimental  
 828 design. The ANOSIM statistic R and the significance values are shown. The proportion of the  
 829 variance explained by each model ( $R^2$ ) and probability (P) are shown.

Origin	Inoculation	Effect of root system			
		Bacteria		Fungi	
		$R^2$	Pr(>F)	$R^2$	Pr(>F)
Arable	Arable	-0.029	0.551	-0.008	0.520
Arable	NotInoculated	0.016	0.381	0.341	<b>0.001</b>
Arable	Woodland	0.005	0.398	0.251	<b>0.015</b>
Control	Arable	-0.080	0.818	-0.075	0.797
Control	NotInoculated	0.172	<b>0.013</b>	0.687	<b>0.012</b>
Control	Woodland	-0.021	0.461	-0.029	0.564
Woodland	Arable	-0.047	0.624	0.019	0.355
Woodland	NotInoculated	0.108	0.121	0.056	0.213
Woodland	Woodland	0.013	0.367	-0.004	0.483
Origin	Root system	Effect of inoculation			
		Bacteria		Fungi	
		$R^2$	Pr(>F)	$R^2$	Pr(>F)
Arable	Fibrous	0.460	<b>0.001</b>	0.709	<b>0.001</b>
Arable	Noroot	0.362	<b>0.003</b>	0.577	<b>0.001</b>
Arable	Tap	0.398	<b>0.004</b>	0.753	<b>0.001</b>
Control	Fibrous	0.616	<b>0.001</b>	0.825	<b>0.001</b>
Control	Noroot	0.501	<b>0.002</b>	0.798	<b>0.001</b>
Control	Tap	0.505	<b>0.002</b>	0.497	<b>0.001</b>
Woodland	Fibrous	0.513	<b>0.001</b>	0.789	<b>0.001</b>
Woodland	Noroot	0.590	<b>0.001</b>	0.612	<b>0.001</b>
Woodland	Tap	0.563	<b>0.001</b>	0.820	<b>0.001</b>

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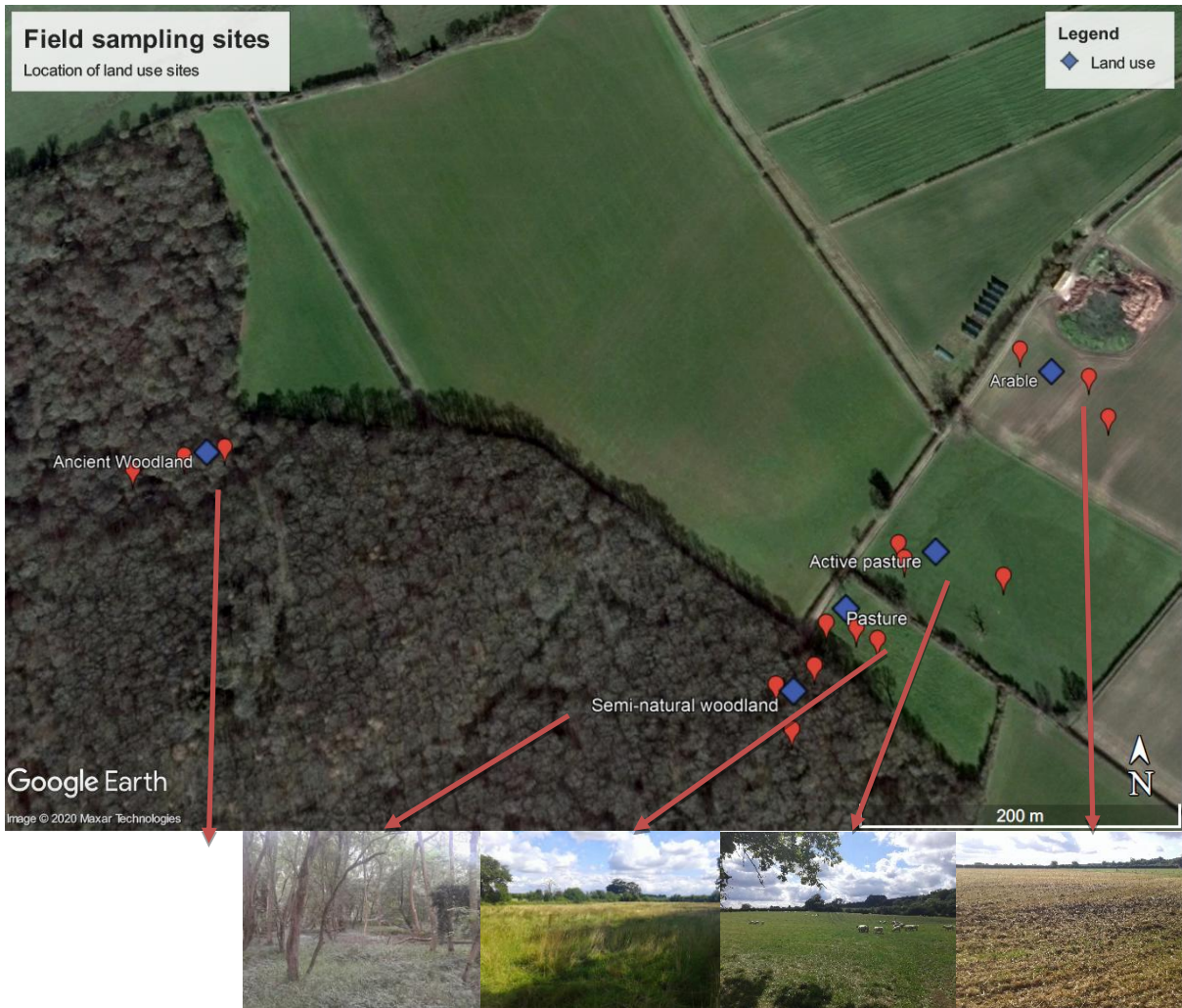


840 **Table 6.** Effects of inoculation, soil origin and root system type and their interactions on soil  
841 aggregate stability assessed with ANOVA. An additional ANCOVA model was tested including root  
842 length density (RLD), to separate the effect of species and physical properties (quantity of roots)  
843 within the effect of root system treatment. The degrees of freedom (Df), sum of squares (Sum of sqs),  
844 mean of squares (Mean of sqs), the F. statistic and p-value (P) associated with the F statistic are  
845 shown.

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Factors	Df	Sums of sqs	Mean of sqs	F.Model	Pr(>F)
Origin	2	45.76	22.881	460.529	<0.001
Inoculation	2	0.33	0.166	3.348	0.043
Root system	2	0.07	0.033	0.656	0.523
Origin*Inoculation	4	1.76	0.439	8.833	<0.001
Origin*Root system	4	0.3	0.076	1.531	0.206
Inoculation*Root system	4	0.06	0.016	0.318	0.865
Origin*Inoculation*Root system	8	0.37	0.046	0.92	0.507
Residuals	54	2.68	0.05		
<b>Model including RLD</b>					
Origin	2	45.76	22.88	871.82	<0.001
Inoculation	2	0.33	0.166	6.343	0.005
Root system	2	0.07	0.033	1.246	0.302
RLD	1	0.04	0.038	1.457	0.237
Origin*Inoculation	4	1.89	0.472	17.994	<0.001
Origin*Root system	4	0.24	0.059	2.264	0.085
Inoculation*Root system	4	0.04	0.01	0.385	0.817
Origin*RLD	2	0.01	0.006	0.22	0.803
Inoculation*RLD	2	0.6	0.301	11.462	0.000
Root system*RLD	2	0.04	0.019	0.707	0.501
Origin*Inoculation*Root system	8	0.38	0.048	1.83	0.109
Origin*Inoculation*RLD	4	0.38	0.096	3.649	0.015
Origin*Root system*RLD	4	0.37	0.092	3.5	0.018
Inoculation*Root system*RLD	3	0.31	0.104	3.955	0.017
Origin*Inoculation*Root system*RLD	5	0.06	0.011	0.43	0.824
Residuals	31	0.81	0.026		

847 **Figures:**



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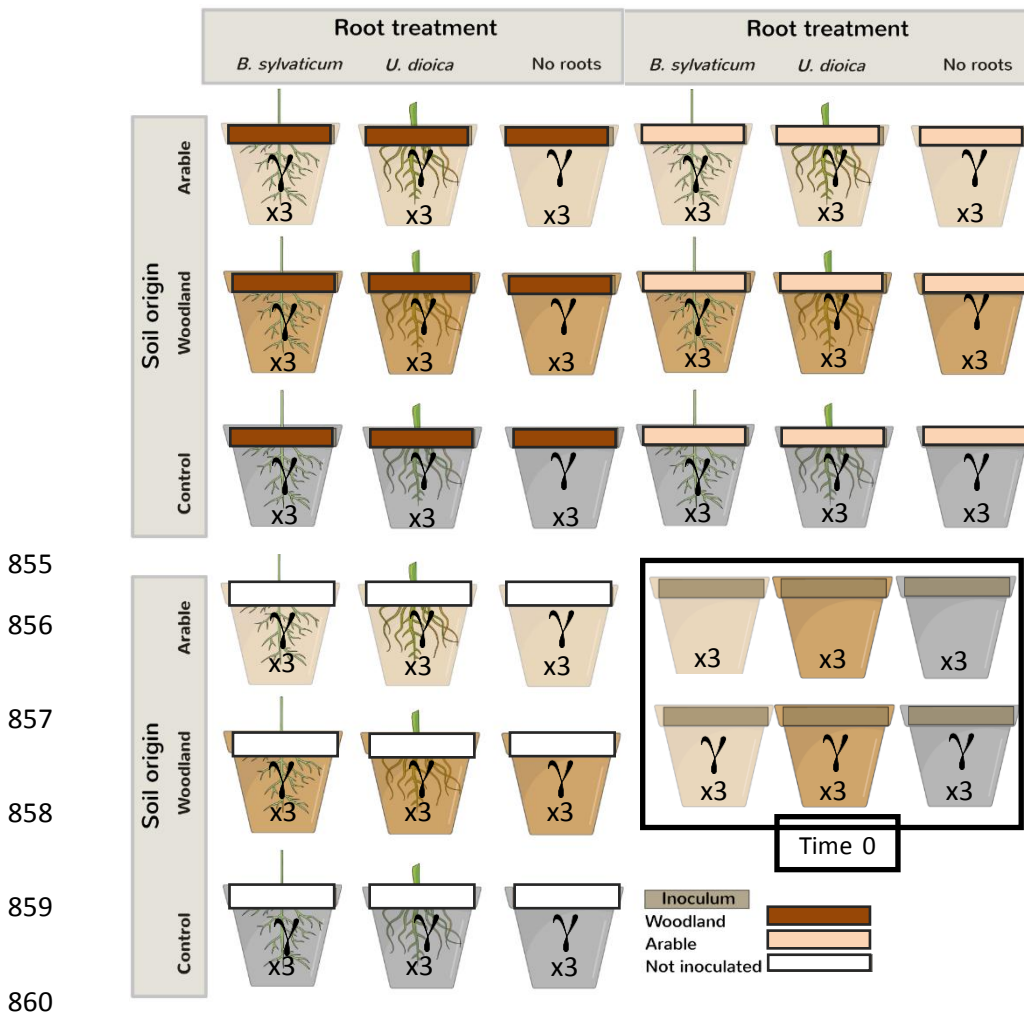
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850 **Figure 1.** Location of sampling plots spanning a range of land uses. Red dots correspond to plots  
851 where sampling was performed. (Source: 51°46'46.91''N and 1°19'35.76''. Google Earth  
852 v7.3.2.5776, May 20, 2018. May 11, 2020).

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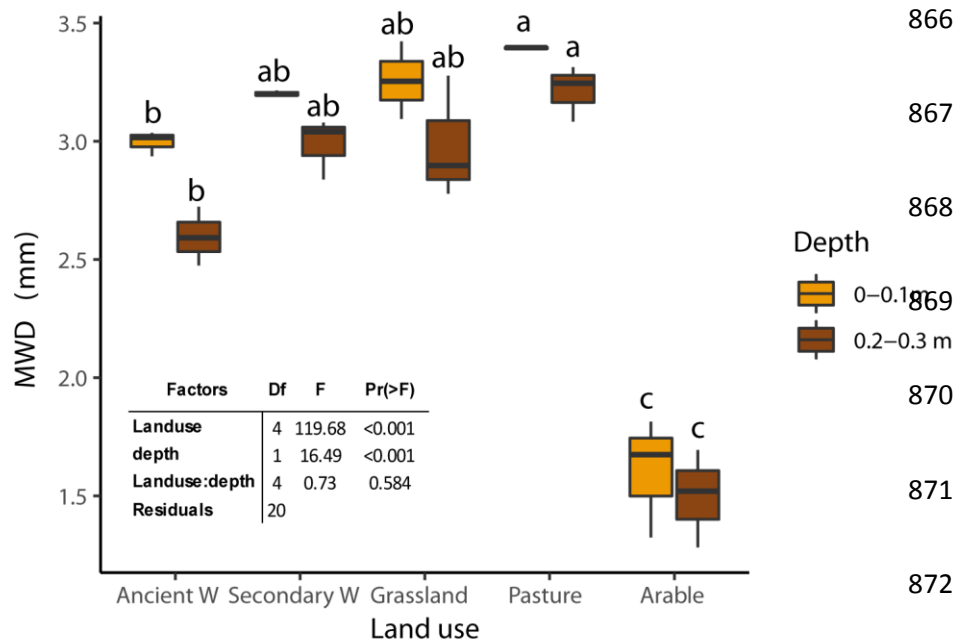


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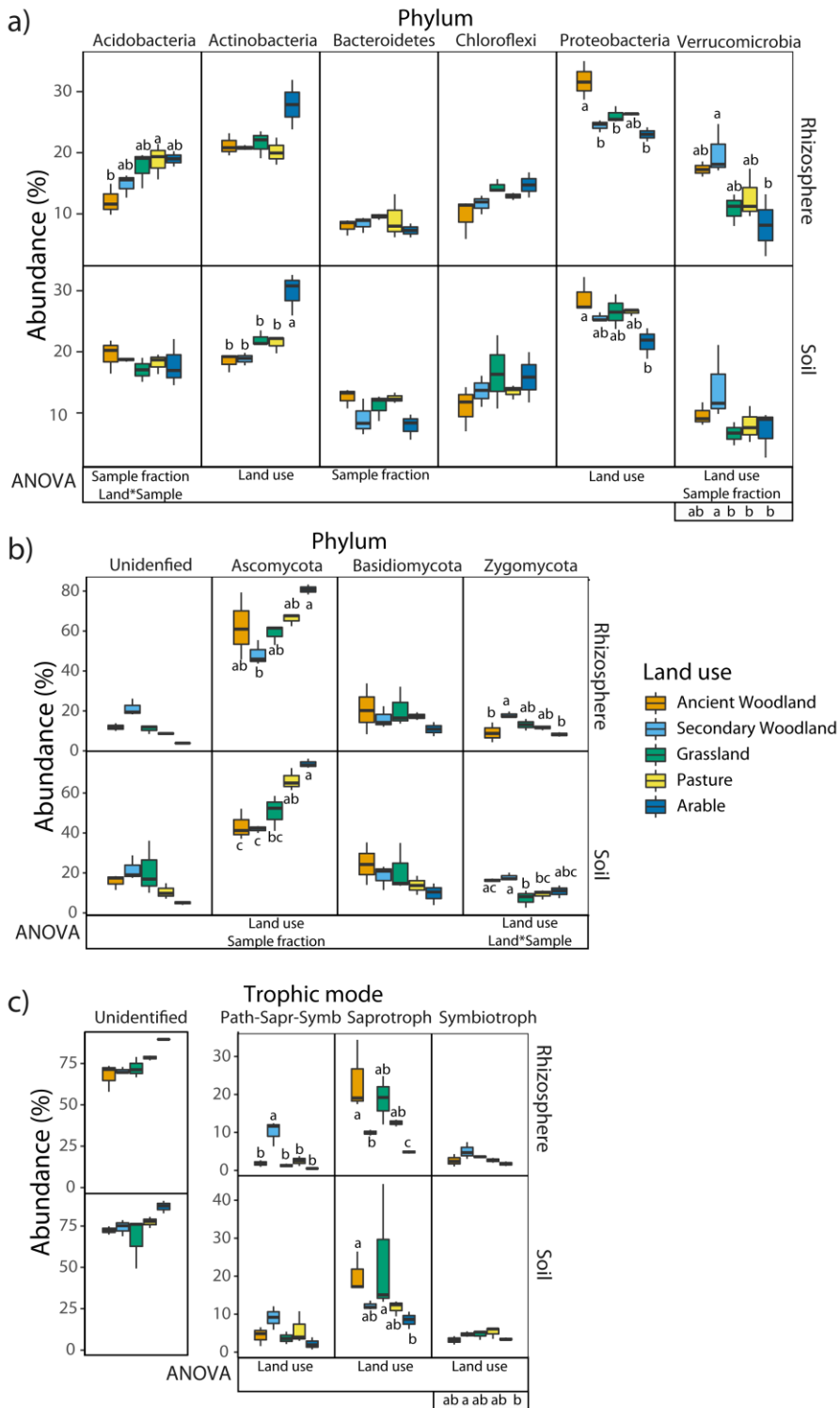
861 **Figure 2.** Schematic illustration of the treatments under study with three soil origins, three root  
862 system types (fibrous root system of *Brachypodium sylvaticum*, taproot system of *Urtica dioica* and a  
863 control with no roots present) and three inoculation treatments. Different coloured bars on tops of pots  
864 refer to the source of soil inoculum (see legend).

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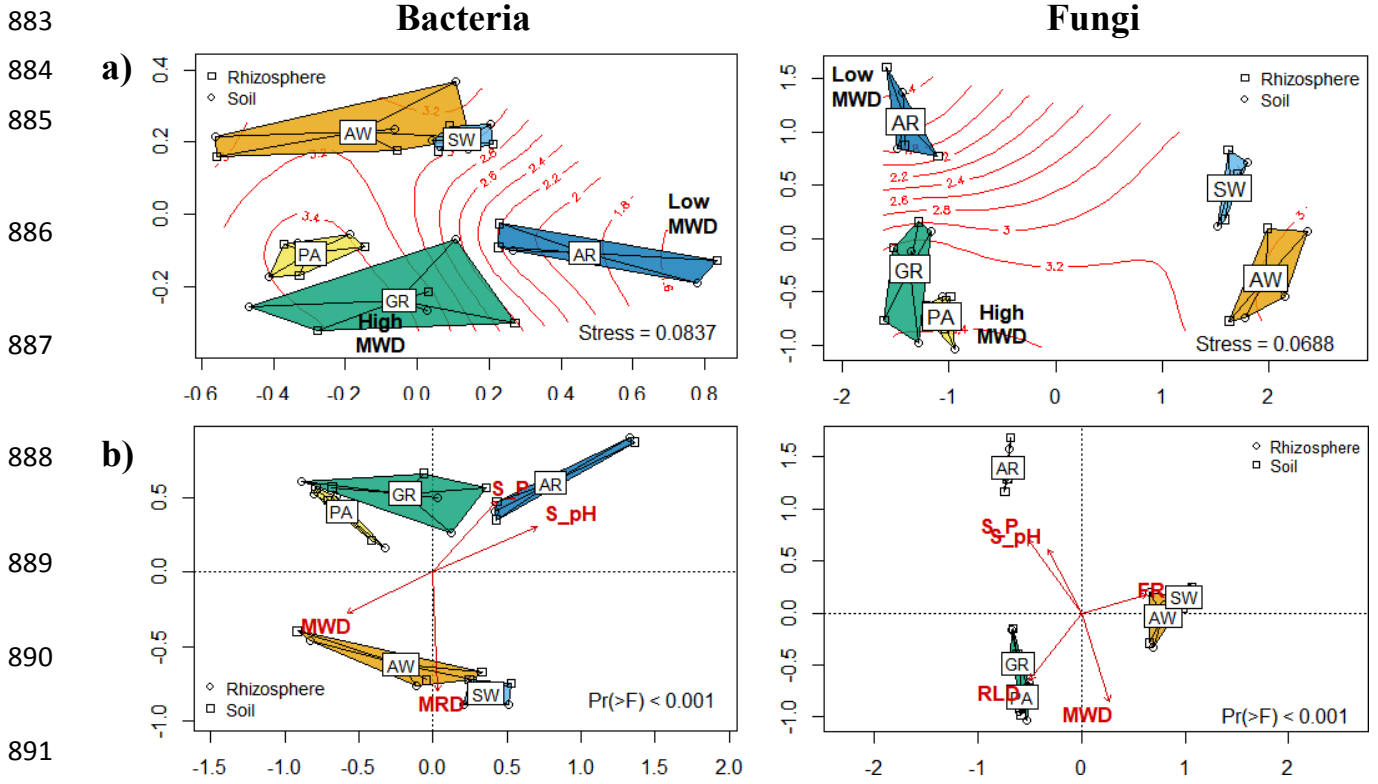


873 **Figure 3.** Soil aggregate stability (MWD) measured in the field in the different land uses at the  
 874 surface and 0.2 – 0.3 m depth. Boxplots represent the minimum, maximum, median, first quartile and  
 875 third quartile in the data set. Different letters show post-hoc Tukey honestly significant difference  
 876 (HSD) results between land uses for the two different depths.

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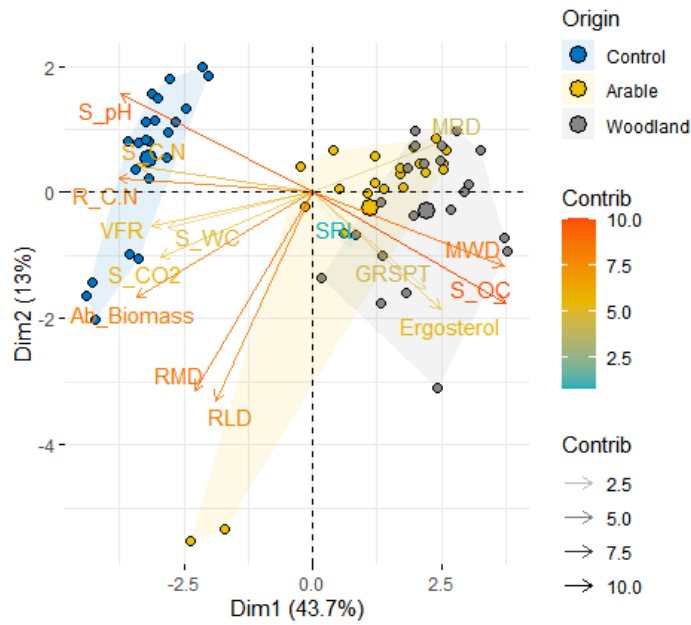
878 **Figure 4.** Boxplots with relative abundance of phyla found in the three plots in each land use in  
 879 rhizosphere and bulk soil fractions for bacteria (a) and fungi (b) and proportion of fungal trophic  
 880 modes (c). Boxplots represent the minimum, maximum, median, first quartile and third quartile in the  
 881 data set. Different letters show post-hoc Tukey honestly significant difference (HSD) results. Letters  
 882 out of the boxplot figure show the overall effect including rhizosphere and bulk soils.



892 **Figure 5.** a) NMDS ordinations of bacteria and fungi species matrices with convex hull polygons and  
 893 spider diagrams containing plots of the five sites and overlaid gradients of aggregate stability and b)  
 894 graphs of dbRDA constrained ordinations of bacteria and fungi species matrices with convex hull  
 895 polygons and spider diagrams containing plots of the five sites and significant variables obtained by  
 896 automatic backward stepwise model building. Data are shown for rhizosphere (□) and bulk soil (○)  
 897 fractions. Acronyms for land use types: AW is ancient woodland, SW is secondary woodland, GR is  
 898 grassland, PA is pasture, and AR is arable. Acronyms for factors: S\_pH is soil pH, S\_P is soil P,  
 899 MWD is mean weight diameter, MRD is mean root diameter, FR is % fine roots, RLD is root length  
 900 density.

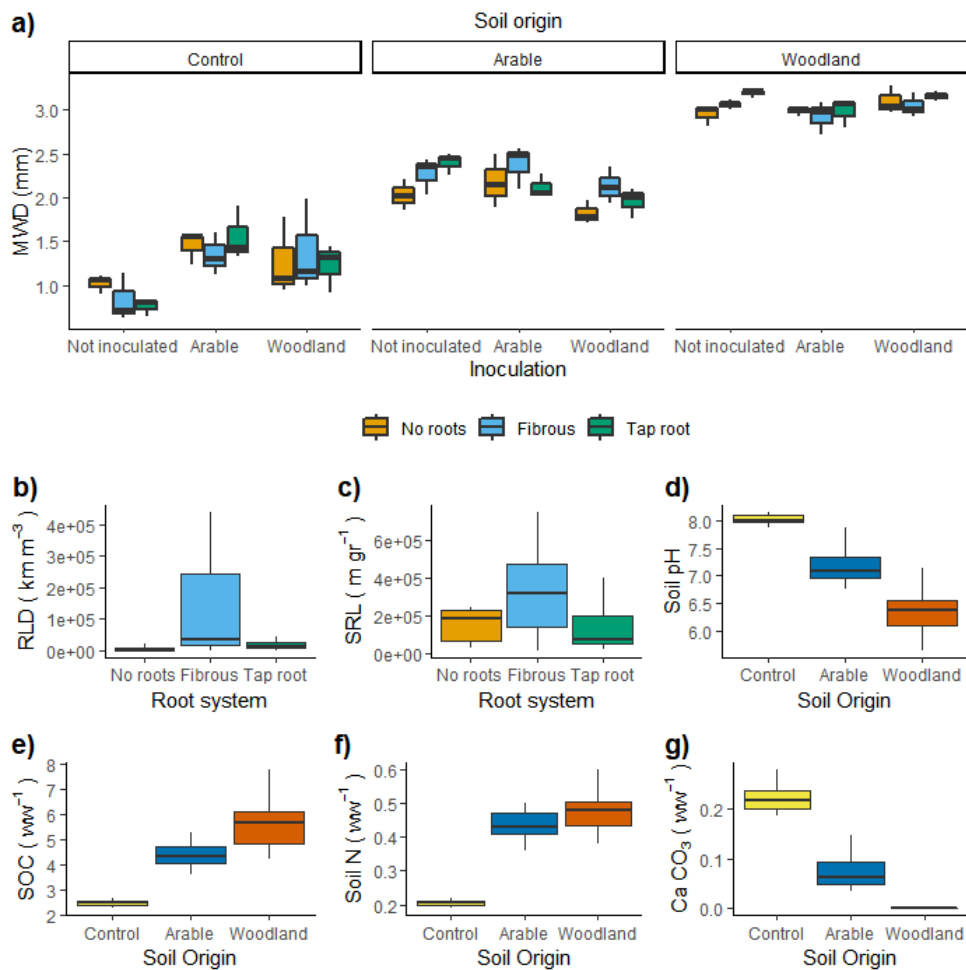
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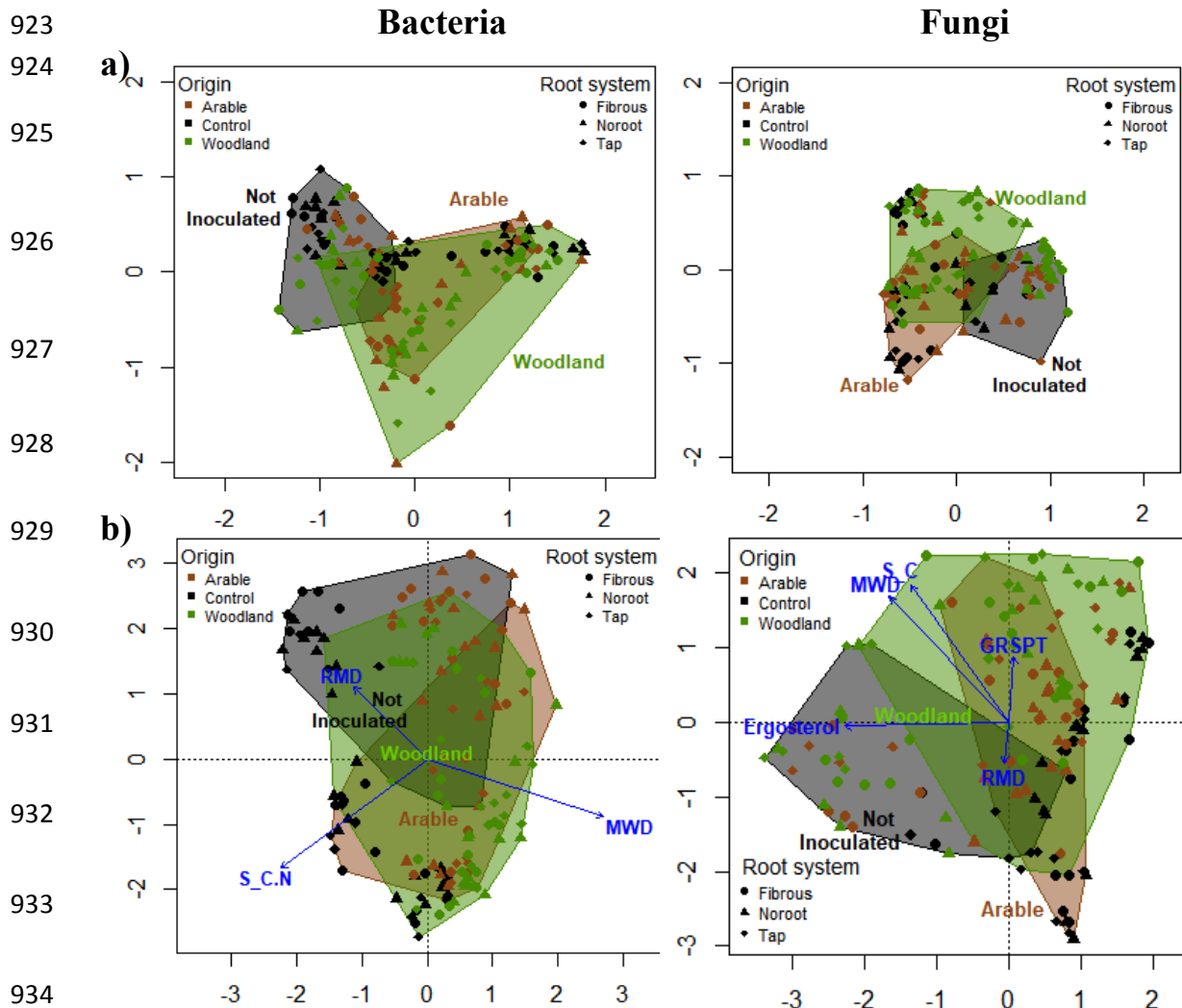
**Figure 6.** PCA of variables used for the description of soil, vegetation and root properties in the experiment. Variables showed were selected based on their correlation and ecological significance (i.e. variables that were correlated but their source was ecologically different were kept, e.g. soil C:N and root C:N). Acronyms: S\_pH is soil pH, S\_C:N is soil C:N ratio, S\_WC is soil water content, S\_CO2 is rate of exchange of CO<sub>2</sub>, S\_OC is soil organic carbon, MWD is mean weight diameter, Ab\_biomass is Aboveground biomass, R\_C:N is root C:N ratio, VFR is % very fine roots, RMD is root mean diameter, RLD is root length density, MRD is mean root diameter, SRL is specific root length, GRSPT is total glomalin related soil proteins.



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918 **Figure 7.** Selected specific traits of soil samples after incubation period: a) soil aggregate stability  
 919 (mean weight diameter, MWD), b) root length density (RLD,  $\text{km m}^{-3}$ ), c) specific root length (SRL,  $\text{m}$   
 920  $\text{gr}^{-1}$ ), d) soil pH, e) soil organic carbon (SOC,  $\text{ww}^{-1}$ ), f) soil nitrogen (N,  $\text{ww}^{-1}$ ), g) soil total inorganic  
 921 carbon ( $\text{CaCO}_3$ ,  $\text{ww}^{-1}$ ). Boxplots represent the minimum, maximum, median, first quartile and third  
 922 quartile in the data set.





935 **Figure 8.** a) NMDS ordinations of bacteria and fungi species matrices with convex hull polygons  
 936 containing plots of the three inoculation treatments (not inoculated, woodland and arable) and b)  
 937 graphs of dbRDA constrained ordinations of bacteria and fungi species matrices with convex hull  
 938 polygons containing plots of the three inoculation treatments (not inoculated, woodland and arable)  
 939 and significant variables obtained by automatic backward stepwise model building. Acronyms are:  
 940 S\_C is soil total carbon, S\_C.N is soil C:N ratio, MWD is mean weight diameter, RMD is root mean  
 941 diameter, GRSPT is total glomalin related soil proteins.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: