BENEFITS OF APPLYING ORGANIC AMENDMENTS FROM RECYCLED WASTES FOR FUNGAL COMMUNITY GROWTH IN RESTORED SOILS OF A LIMESTONE QUARRY IN A SEMIARID ENVIRONMENT

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1 **Abstract**

2 Applying organic amendments to recover physical, chemical, and biological qualities of
3 soil may enable recovery of soils degraded by mining in semiarid climates. This study's
4 aim was to investigate the development and changes in the composition of fungal
5 communities in restored soils with five different types of organic amendments (two types
6 of vegetable compost and sewage sludge compost, and a mixture of both) compared with
7 unamended soils and surrounding natural soils and to examine the relationships between
8 the fungal taxa, the new physico-chemical and biological soil properties of technosoils
9 after 18 months of restoration, and natural soils. Restoration improved soil quality and
10 fungal diversity, placing these soils in an intermediate position between unrestored soils
11 (with no fungi present) and undisturbed reference soils, which were the most fungal
12 diverse. Sewage-treated soils and their mixtures showed high nitrogen and carbohydrate
13 content as well as high basal respiration and fatty acid content, suggesting that they
14 provided readily biodegradable organic matter. In contrast, greenhouse compost-treated
15 soils showed high total organic carbon and polyphenol content, whereas garden compost-
16 treated soils showed intermediate values. The biological soil properties of both composts
17 showed were similar to those of the reference soils, suggesting that composts contained
18 more resilient organic matter. Organic amendments of dissimilar origin caused
19 significantly different fungal soil communities at the genus level among the restored soils.

Results indicated that soil pH, electrical conductivity, total nitrogen content, soil basal respiration, fungi/bacteria-PLFA ratio, and dehydrogenase and β-glucosidase activities, together with Pearson's correlations, revealed that these properties and nutrient content (total organic carbon, C/N ratio, carbohydrates, and polyphenols) influenced 40 soil fungal taxa. Therefore, the organic amendments led to changes in soil properties that favoured plant cover by promoting the soil fungal community growth beneficial to the carbon cycle and symbiotic with plants.

Keywords: Degraded area, Fungal community, Limestone quarry, Microbial activity, Organic amendments, Soil restoration.

1. Introduction

Mining activity has induced the degradation of 800,000 km^2 globally (Cherlet et al., 2018), severely affecting soil quality and significantly reducing the abundance and diversity of soil biota (Ohsowski et al., 2012). In particular, opencast mining presents a major ecological threat, because it removes the soil, changing its physical, chemical, biochemical, and biological soil properties and thus altering the plant community (García-Ávalos et al., 2018; Luna et al., 2017; Song et al., 2020; Soria et al., 2021a) by directly disturbing the microbiological communities that inhabit it (Chen et al., 2020; Rodríguez-Berbel et al., 2021, 2020). Degraded ecosystems, especially those located in arid and semiarid regions, require ecological restoration for their recovery, given that the removal of the organic substrate together with adverse climatic conditions (water stress, high temperatures, erosion, etc.; Luna et al., 2016; Miralles et al., 2012) and the low organic C content that characterises these soils (Bastida et al., 2016; Pascual et al., 1997) cause changes in physical, chemical, and biological soil properties that hinder the natural regeneration of plant cover and microbiota in these soils (Ferrol et al., 2004; Ricks and Koide, 2019).

The success of a restoration depends on the integration of strategies aimed at optimising the interactions between soil components and improving soil quality (Requena et al., 2001). Many authors have proposed the potential application of organic amendments for restoring of semiarid soils because such amendments help improve physical, chemical, biochemical, and biological soil properties (Almendro-Candel et al., 2014; Luna et al., 2016; Peñaranda Barba et al., 2020; Ros et al., 2003). Moreover, the use of these remains could provide an alternative to the accumulation and/or incineration of biodegradable

52 waste (Almendro-Candel et al., 2014; Debosz et al., 2002), thus contributing to the
53 circular economy through resource conservation and waste recycling (Hueso-González et
54 al., 2018). Differences in the nature of the organic amendments provides different
55 characteristics in the organic materials that compose them (labile or resilient C), which,
56 together with the environmental parameters (temperature, humidity, climate, etc.),
57 determines the availability of nutrients (C, N, and P) in the soils (Bastida et al., 2017,
58 2008; González-Ubierna et al., 2012; Pérez-Gimeno et al., 2019; Ros et al., 2003). The
59 microbial community's efficiency in adapting to resource consumption (Guo et al., 2018)
60 could have a differential effect on soil microbiota by influencing the microbial use of C
61 contained in these materials (Martens, 2000), which could generate changes in the
62 development of some microbial taxa versus others better adapted to the applied
63 amendment (Bastida et al., 2008). However, the changes produced in soil properties
64 (physical, chemical, nutrient content, etc.) following the application of organic
65 amendments and their impact on soil microbiological communities have not yet been fully
66 explored (Li and Wu, 2018; Paula et al., 2020).

67 Soil microbial diversity is critical for maintaining soil fertility and functionality (Xue et
68 al., 2017), as microorganisms regulate most soil biological processes (Nannipieri et al.,
69 2017) and biogeochemical cycles (Fierer, 2017; Li and Wu, 2018). Several authors have
70 pointed out the soil microbial community as an important indicator of soil quality (Xue
71 et al., 2020; Zak et al., 2003). The addition of organic amendments induces
72 microorganisms to respond differently to changes in edaphic properties (pH, EC,
73 nutrients, etc.) as well as in basal respiration, fatty acid profile and enzymatic activities
74 (Bastida et al., 2016, 2008; Dick, 1997; Torres et al., 2016). Most existing studies focus
75 on the behavioural response of bacterial communities to these perturbations (Lauber et
76 al., 2009; Zhao et al., 2021), whereas fungal community behaviour has been poorly
77 monitored in restored ecosystems (Guo et al., 2018; Hart et al., 2019). Fungi are key links
78 in the physical, chemical and biological soil properties (Hart et al., 2019), as they
79 participate in nutrient cycling, organic matter (OM) decomposition and benefit the
80 development of vegetation cover (Boer et al., 2005; Gil-Martínez et al., 2021; Yang et al.,
81 2019). The study of the soil fungal community is of great interest, given that fungi are
82 considered generalist organisms capable of degrading recalcitrant OM (Paula et al., 2020)
83 participating in carbon recycling (Kabel et al., 2020) and allowing microbial succession
84 to exist (Paterson et al., 2008). Therefore, the consideration of soil fungal communities is

an important aspect for the successful restoration of a limestone extraction mine in a semiarid climate.

Previous studies performed 6 months after the application of organic amendments in the same experimental plots revealed that sewage-treated soils showed higher rates of microbial activity (priming effect (PE), basal respiration (BR), and enzymatic activity) indicating a rapid consumption of labile OM, whereas in soils treated with both vegetable compost the microbial activity was lower, indicating, through the soil microbial diversity, an OM more difficult to degrade (Rodríguez-Berbel et al., 2021; Soria et al., 2020, 2021a, 2021b). For this reason, this work describes the study of growth of soil fungal communities in restored soils using amendments from different types of organic wastes compared with surrounding undisturbed reference soils and unrestored soils after 18 months after the restoration of a limestone quarry in a semiarid climate. For this purpose, the following were studied: i) the effect of the application of organic amendments on the physico-chemical and biological soil properties of the restored, control, and natural soils; ii) diversity and composition at the lowest possible classification level of fungal communities in the different soils; iii) determination of the most influential factors in the proliferation of soil fungal taxa; iv) identification of relevant soil fungal taxa in the soils considered; and v) relationships between the physico-chemical and biological soil properties and the soil fungal taxa previously identified. We hypothesised that fungal communities at phylum and genus level would respond differently to each type of treatment applied according to changes in physico-chemical and biological soil properties and in the chemical nature of the C added. Furthermore, we hypothesised that the addition of amendments would accelerate the recovery of soil fungal communities of restored soils towards reference conditions compared with experimental plots that received any treatment. We expect that as communities progress through succession (Morriën et al., 2017), long-term increases in soil microbial biomass and microbiological activity would occur (Debosz et al., 2002).

2. Material and methods

2.1. Study design and sampling

The analyses of this field experiment were taken in December 2019 after a year and a half of restoration (beginning July 2018) in a limestone quarry in the Sierra de Gádor in the province of Almería (SE Spain, 36°55′20″N, 2°30′29″W) in a semiarid climate. The

117 average annual rainfall, mainly occurring in winter and autumn, is 242 mm yr^{-1}, and the
118 mean annual temperature is 17.6ºC. In areas adjacent to the study area that have not been
119 disturbed, shallow soils overlying limestones and dolomites are found with calcareous
120 sandstones and marly, as well as loamy marls that form Regosols (FAO-IUSS-ISRIC
121 Working Group WRB, 2015). The substrate in the experimental study area consists
122 predominantly of calcareous sandstones overlaid on partially extracted marls. In the study
123 area, the native vegetation is composed principally of grassland populated by *Stipa*
124 *tenacissima* (L.) Kunth., *Anthyllis terniflora* (Lag) Pau. and *A. cytisoides* L., among other
125 species. More information about the study area is found in Luna et al. (2016) and Soria et
126 al. (2021a).

127 Fifteen experimental plots of 50 m^2 (10 m × 5 m) each were installed on a flat site at 362
128 m.a.s.l. fully exploited by mining activity. Before installation, heavy machinery was used
129 to homogenise and decompact the soil. To compare the effect of organic amendments
130 with different chemical composition on restored soils, different organic amendments were
131 selected and applied to these experimental plots (3 replicates per treatment) for the soil
132 restoration process. The composts from different plant residues delivered resilient soil
133 OM with a greater contribution of lignin (Argyropoulos and Menachem, 1997) and
134 humic-type polymers (Stevenson, 1994). Stabilised sewage sludge was employed because
135 it contained a higher amount of labile OM constituted principally of proteins, free
136 carbohydrates and a large amount of condensed lipids (Almendros et al., 2000, 1990).
137 Last, mixtures of amendments were combined with the aim to obtain a balanced chemical
138 composition between resilient and labile of OM and to replace nitrogen losses during the
139 composting process with the extra N supplied by the sewage sludge (Shou et al., 2019).
140 The treatments applied were arranged as follows: a) 100% vegetable compost derived
141 from garden waste (CG); b) vegetable compost derived from greenhouse crop residues
142 (CC); c) sewage sludge treated with anaerobic mesophilic digestion, dehydrated by spin
143 and thermally dried at 70ºC (SS); d) mix equal to CG + SS (Mix1); and e) mix equal to
144 CC + SS (Mix2). The amount of each organic amendment applied was estimated to
145 increase the initial soil OM content to 3% in each plot. Then, the organic residues were
146 spread over the soil surface with a shovel backhoe (1 m^3) and mixed with the first 20 cm
147 of mining degraded soils with a bulldozer. Additionally, natural reference soils (NAT)
148 near the experimental plots were taken as reference soils.

149 After installation and application of the organic amendments in the experimental plots,
150 two Mediterranean native species (40 plants of *Stipa tenacissima* L. and 10 plants of *Olea*
151 *europaea* L. var. *sylvestris* Brot.) with high survival rates in previous ecological
152 restorations performed in the study site were selected (Luna et al., 2017). These species
153 were planted by hand, at the distance of 100 cm between plants, from forest pot seedlings
154 (50 plants in total per plot). At the time of planting, a stabilisation irrigation was carried
155 out because of the climatic conditions of the study area and the low rainfall rate. This
156 practice had been used in previous ecological restorations in the same quarry, resulting in
157 a high survival rate of *Stipa tenacissima* L. (Luna et al., 2017). A more detailed
158 description of the construction of experimental plots can be found in Rodríguez-Berbel et
159 al. (2021). After 18 months of organic amendment application, composite soil samples
160 (mixing 10 subsamples) were collected at random from each experimental plot to a depth
161 of 10 cm to study the changes in physico-chemical and biological soil properties and soil
162 fungal communities over the medium term in restored areas of open mines in semiarid
163 climate. Isothermal bags were used to transport a total of 21 soil samples (3 replicates per
164 treatment) to the laboratory. The samples were air-dried, homogenised, sieved (ø 2 mm)
165 and stored at 4ºC to analyse different physico-chemical parameters of the soil. A portion
166 of this dried soil was stored at -20°C for DNA extraction and next generation sequencing
167 (NGS) analysis.

168 The evolution of vegetation cover in the experimental area was studied by conducting
169 three different sampling campaigns at 6 months (November 2018; C1), 12 months (June
170 2019; C2) and 24 months (June 2020; C3) after restoration. The percentage of total
171 vegetation cover area occupied by wild and spontaneous plant colonisation was recorded
172 in each experimental plot (n = 18), as well as the identification of spontaneous vegetation.

173 *2.2. Physical and chemical soil properties*

174 Different physico-chemical soil parameters were studied: i) soil pH was determined on a
175 soil water suspension (1:2.5 soil/water ratio) (Thomas, 1996) with a pHmeter (LAQUA
176 PH1100, HORIBA, Tokio, Japan); ii) electrical conductivity (EC) was measured in an
177 aqueous suspension 1:2.5 soil/water with a digital conductivity metre (LAQUA EC1100,
178 ORIBA, Tokio, Japan); iii) total organic carbon (TOC) was determined by rectified
179 method of Walkey and Black (1934) (Mingorance et al., 2007); iv) total nitrogen content
180 (TN) was measured by total combustion (Vario Rapid N; Elementar, Hanau, Germany);

181 v) C/N ratio was determined from TOC and TN; vi) carbohydrate content (CH) was
182 quantified from a cold extraction (25ºC) on a soil suspension (1:10 soil/water ratio) for
183 1h under agitation using the anthrone–sulphuric acid method (Brink et al., 1960); and vii)
184 polyphenol content (POL) were measured using Folin-Ciocalteau reagent by the Folin–
185 Denis method (Ribéreau-Gayon and Gautheret, 1968). CH and POL absorbance
186 measurements were performed with a spectrophotometer, Spectronic Helios Gamma UV-
187 Vis (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

188 *2.3. Soil basal respiration, fatty acids and enzymatic activity*

189 The following biological soil properties were analysed: i) soil basal respiration (BR) was
190 measured from 20 g of sample at 50% water holding capacity in 125 ml hermetically
191 sealed vials that were incubated for 31 days (28ºC in darkness), and the CO_2 produced
192 was periodically measured (24 h, 48 h and 72 h and then each 4 days) using an infrared
193 gas analyser (CheckmateII; PBI Dansensor, Ringsted, Denmark) (Soria et al., 2021a); and
194 ii) ester-linked fatty acid methyl esters (FAMEs), hereafter fatty acids, were extracted
195 from 3 g of soil (Schutter and Dick, 2000). Fatty acids were analysed with a Trace Ultra,
196 Thermo Scientific gas chromatograph fitted with a 60 m capillary column (SGE
197 Analytical Science, BPX70, 60 m x 0.25 mm ID x 0.25 μm film) using helium as the
198 carrier gas. Conditions were as follows: i) initial temperature of 120°C for 30s, increased
199 to 140 °C with a ramp of 1 °C/min, then to 170 °C with increments of 2 °C/min, and
200 finally to 210 °C at 2 °C/min; ii) the fatty acids i15:0, 15:0, a15:0, i16:0, i17:0, 16:1ω9,
201 cy17:0, cy19:0, 10Me16:0, and 10Me18:0 were accounting for the bacterial biomass (B-
202 PLFA; Dungait et al., 2011; Frostegård et al., 1993); iii) the phospholipid fatty acids
203 18:2ω6 were predictors of the fungal biomass (F-PLFA; Bastida et al., 2019; Rinnan and
204 Bååth, 2009); iv) fungus/bacteria ratio calculated with the two previous ones (F/B-PLFA
205 ratio); v) the fatty acids 15:1, 16;1ω7, 15:1ω6, 16:1ω5, 17:1, 18:1ω9c, 18:1ω7 and
206 18:1ω9t represent monounsaturated fatty acid (M-PLFA); vi) the fatty acids 14:0, i15:0,
207 a15:0, 15:0, i16:0, 16:0, i17:0, cy17:0, 17:0, 18:0, 20:0, 22:0 and 24:0 represent saturated
208 fatty acids (S-PLFA); and, vii) The ratio of M-PLFA-to-S-PLFA is expressed as M/S-
209 PLFA ratio.

210 Similarly, the enzymatic activities were studied using 1 g of sample as follows: i)
211 Dehydrogenase activity (DHA) was analysed (García et al., 1997); ii) β-glucosidase

212 activity and iii) urease activity were determined according to published methods (Eivazi
213 and Tabatabai, 1988; Kandeler and Gerber, 1988).

214 *2.4. DNA isolation, PCR amplification, sequencing and bioinformatics analysis*

215 Microbial DNA was extracted from 0.3 g of soil sample using a PowerSoil DNA Isolation
216 kit (QIAGEN Inc., Germany), following the manufacturer's instructions. A ND-2000
217 Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) was used to quantify the
218 DNA concentration (ng/µl). To characterise fungal community composition, fungal *ITS*
219 genes were amplified and sequenced by polymerase chain reaction (PCR) using the
220 primer pairs ITS86F/ITS4 (Sommermann et al., 2018). To verify no contamination
221 throughout the DNA extraction, one blank control was added using one of the kit tubes.
222 Also, negative controls were tested (1 for every 96-well plate = 4 per MiSeq run) and
223 checked to be clean (no bands present) and still sequenced on the MiSeq to show that no
224 substantial reads were generated on this barcode combination.

225 The final sequence files were then processed using QIIME2 software (version 19.10)
226 (Bolyen et al., 2018) following the protocol on the Microbiome Helper website
227 (Amplicon SOP v2 [qiime2 2019.7];
228 https://github.com/LangilleLab/microbiome_helper/wiki (Comeau et al., 2017)).
229 Taxonomy assignments of fungal phylotypes were performed in reference to the UNITE
230 database (version 7). Diversity statistical measures were calculated in QIIME2 using the
231 ASV table normalised to 10,000 sequences per sample. This procedure provides as a
232 result an abundance table with taxonomy information, which was then analysed and
233 visualised using the online web tool Calypso (Zakrzewski et al., 2017). From this tool,
234 the relative abundance of fungal taxa was calculated, and those fungi with an abundance
235 greater than 0.1% in each type of organic amendment (n = 3) and in all samples (n = 21)
236 were selected.

237 *2.5. Statistical analysis*

238 Statistical differences on physico-chemical and biological soil parameters of each
239 experimental plot (CG, Mix1, SS, Mix2, CC and NAT) were analysed by one-way
240 univariable and multivariate permutational analysis of variance (PERANOVA and
241 PERMANOVA (Anderson, 2001), with 9999 perms, $p < 0.05$), using Euclidean and
242 Bray–Curtis distance similarity matrices, respectively. A pairwise test comparison by
243 permutation was performed to construct a multivariate analogue of the *t* test and the

244 probability levels of differences between groups (Eldridge et al., 2016); a Monte-Carlo
245 test was performed when the number of free permutations was less than 100.

246 Relationships between changes in the fungal community structure and individual soil
247 characteristics were performed by distance-based linear modelling (DistLM; Anderson et
248 al., 2008), confronting the relative abundance of soil fungal taxa, first, with the physico-
249 chemical soil variables and, second, with the biological soil variables analysed. Last,
250 distance-based redundancy analyses (dbRDA) (based on the Bray–Curtis dissimilarity)
251 were used to estimate the relative abundance of fungal taxa caused by each of the selected
252 soil properties, and marginal tests were performed to test the significance of each test
253 (McArdle and Anderson, 2001). The Akaike Information Criterion (AICc) criterion was
254 used for selecting the best model, and the stepwise approach for building the model was
255 followed (Akaike, 1974). The statistical package PRIMER6 + PERMANOVA software
256 (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) was performed for PERMANOVA,
257 PERANOVA and dbRDA analysis.

258 R Project environment (R Core Team, 2018) with 'corrplot' and 'RColorBrewer'
259 packages by Pearson's correlation (r; $p < 0.05$) was used to generate a heatmap plot to
260 assess the relationships between soil properties and identified soil fungal taxa. The linear
261 discriminant analysis (LDA) effect size (LEfSe) method by Calypso was utilised to
262 identify soil fungal taxa specific to each restoration treatment and natural soils.

263 **3. Results**

264 *3.1. Physico-chemical soil parameters*

265 Organic amendments improved the physico-chemical conditions of the restored soils
266 (CG, SS, CC, Mix1 and Mix2), increasing nutrient content (TOC, TN, CH and POL) and
267 salinity (EC) and decreasing soil pH compared to unrestored soils (CON; Table 1). SS-
268 treated soils followed by their mixtures (Mix1 and Mix2) showed significantly higher (p
269 < 0.05) EC, TN and CH and lower soil pH and C/N ratio than unamended soils (CON)
270 and reference soils (NAT; Table 1). Soils treated with greenhouse compost (CC) showed
271 significantly higher values ($p < 0.05$) of TOC and POL than CON and reference soils
272 while showing no significant differences in soil pH with the previous ones (Table 1). The
273 CON and reference soils had significantly lower values of TOC, TN, CH and POL and
274 higher C/N ratio values, while the CH and POL content in CON soils was zero (Table 1).

275 The CG-treated soils presented intermediate values between the rest of the restored soils
276 and the reference soils (CON and NAT) for most of the soil properties studied (Table 1).

277 *3.2. Biological soil characteristics*

278 After 18 months of organic amendment application, the biological soil properties (BR and
279 PLFA) and enzymatic activities (DHA, β-glucosidase and urease) of the restored soils
280 (CG, SS, CC, Mix1 and Mix2) increased with respect to the control soils (CON),
281 approaching the values of the reference soils (NAT; Table 2). CON soil showing
282 significantly lower values ($p < 0.05$) for most of parameters analysed (BR, F-, B-, S- and
283 M-PLFA, DHA and β-glucosidase activities; Table 2). BR values were significantly
284 higher ($p < 0.05$) in SS-treated soils followed by their mixtures with compost (Mix1 and
285 Mix2; Table 2). Soils treated with CG and CC showed no significant differences ($p <$
286 0.05) in BR with reference soils, showing intermediate values between CON and soils
287 treated with SS (SS, Mix1 and Mix2; Table 2). The F-PLFA content of the SS-treated
288 soils was similar to that shown by reference soils, while CG-treated soils exhibited
289 significantly lower values ($p < 0.05$) than the previous and significantly higher ($p < 0.05$)
290 than the CON soils. B-PLFA content was significantly higher ($p < 0.05$) in SS soils, while
291 their mixtures (Mix1 and Mix2) and CC-treated soils did not show significant differences
292 with reference soils (Table 2). On the other hand, the F/B-PLFA ratio was higher in
293 reference soils followed by CC soils, with the mixtures and the CON soils presenting
294 intermediate values between the previous whereas SS and CG soils showed the lowest
295 values (Table 2). The content of S- and M-PLFAs was significantly higher ($p < 0.05$) in
296 SS soils than in the rest of the soils (restored, natural and unrestored soils), whereas the
297 ratio between the above (S/M-PLFA ratio) did not show significant differences between
298 restored, CON, and reference soils, despite showing the highest values in SS and Mix1
299 soils (Table 2).

300 As for enzyme activity (DHA, β-glucosidase and urease), restored soils showed
301 intermediate rates between CON soils (significantly lower; $p < 0.05$) and reference soils
302 (higher values), except for DHA activity, where CC-treated soils showed the highest
303 significant values ($p < 0.05$), followed by reference and SS-treated soils (Table 2). β -
304 glucosidase and urease activities rates were significantly higher ($p < 0.05$) in reference
305 soils (NAT), followed by SS and Mix2 soils (Table 2).

306 *3.3. Fungal soil diversity and community composition*

307 *3.3.1. Fungal taxa in organic amendments before application*

308 From taxonomic analysis at phylum level of the organic amendments previous to their
309 application, only Ascomycota was identified at around 10% in each amendment (CG_0,
310 CC_0 and SS_0), whereas at the genus or the next available higher taxonomic level, seven
311 fungal taxa were identified. In the CG amendment, *uncultured* (Class: Eurotiomycetes)
312 and *Phialosimplex* were the most abundant fungal taxa, whereas in the SS amendment,
313 the most abundant were *Microascus* and *uncultured* (Order: Eurotiales). Lastly, in the CC
314 amendment, the most abundant fungal taxa were *uncultured* (Fam: Plectosphaerellaceae)
315 and *Sodiomyces* (Table S2).

316 *3.3.2. Richness and diversity indices in restored and natural soils*

317 Restored soils (CG, SS, CC, Mix1 and Mix2) showed lower values for diversity indices
318 with respect to reference soils (NAT; Table 3) but were higher than CON soils, given that
319 the latter did not pass the quality parameters of the bioinformatic analysis, so they were
320 excluded from the diversity analysis and the rest of the biological analysis (Table S1).
321 CG-treated soils presented values significantly similar ($p < 0.05$) to those of reference
322 soils in Pielou and Shannon indices, whereas in the richness of ASVs, they showed
323 significantly higher values ($p < 0.05$) than the rest of the amended soils (Table 3). SS-
324 treated soils showed lower values than the rest of the soils, being significantly lower ($p <$
325 0.05) for Shannon index (Table 3). The CC soils and mixtures (Mix1 and Mix2) showed
326 intermediate values between the CG and SS soils, with Mix2 standing out in ASV richness
327 and the CC and Mix1 soils in both diversity indices (Pielou and Shannon; Table 3).

328 *3.3.3. Fungal soil community in restored and natural soils*

329 The soil fungal community did not show significant differences (PERMANOVA; $p <$
330 0.05) at phylum level between restored soils (CG, SS, CC, Mix1 and Mix2) but did differ
331 significantly ($p < 0.05$) from reference soils (NAT; Figure S1). Four phyla (Ascomycota,
332 Basidiomycota, Chytridiomycota and Mortierellomycota), along with one Unclassified
333 phylum, were identified with an abundance greater than 0.1% of the total. The most
334 abundant phylum in all soils (restored and reference soils) was Ascomycota, although it
335 presented lower relative abundance in reference soils than in the rest of the treatments,
336 while the Unclassified phylum was mainly recorded in reference soils (Figure S1).

337 Statistical analysis (PERMANOVA; $p < 0.05$) of the f soil ungal community at genus or
338 the next available higher taxonomic level showed significant differences in relative
339 abundances between restored soils (CG, SS, CC, Mix1 and Mix2) and natural soils
340 (NAT), whereas no difference was evident between SS-treated and Mix2 soils (Table S2).
341 Fifty-seven genera were identified with a relative abundance higher than 0.1% of the total.
342 In CG-treated soils, the most abundant soil fungal taxa were *Botryotrichum*, *uncultured*
343 (Fam: Nectriaceae) and *Alternaria*, while in CC soils were *uncultured* (Fam:
344 Microascaceae), *uncultured* (Fam: Plectosphaerellaceae) and *uncultured* (Fam:
345 Nectriaceae) (Table S2). The SS soils had high relative abundances of the genera
346 *Microascus*, *Gymnascella* and *Lophotrichus*, whereas in Mix1 the most abundant fungal
347 genera were *Cephaliophora*, *Microascus*, *Botryotrichum* and *Acremonium*, and in the
348 Mix2 soils the most abundant were *Microascus*, *uncultured* (Fam: Microascaceae) and
349 *Acremonium* (Table S2). Finally, the most abundant fungal taxa in the reference soils
350 (NAT) were Unclassified, *Exophiala* and *uncultured* (Class: Dothideomycetes) (Table
351 S2).

352 *3.4. Relationship between soil characteristics and fungal soil taxa*

353 *3.4.1. Fungal taxa and physico-chemical soil properties*

354 Redundancy analysis in combination with sequential tests showed that the best linear
355 distance model ($R^2 = 0.61$; AICc = 126) was explained by physico-chemical soil
356 properties of pH, EC and TN, the crucial soil variables influencing the soil fungal
357 community (Table S3). The first two components of the dbRDA axes explained 59.98%
358 of the variation in the relationship between soil fungal composition and the physico-
359 chemical parameters studied (Figure 1A). The dbRDA results clearly clustered two
360 different groups: restored soils (CG, CC, SS, Mix1 and Mix2) and reference soils (NAT).
361 Furthermore, it showed that the fungal community of the restored soils (CG, SS, Mix1
362 and Mix2) was related to TN content and salinity, whereas CC-treated soils were related
363 to pH (Figure 1A). The community of reference soils was not related to any physico-
364 chemical factors (Figure 1A).

365 *3.4.2. Fungal taxa and soil basal respiration, PLFA, and enzyme activity*

366 The relationship between soil fungal community and biological soil parameters and
367 enzymatic activity was calculated by dbRDA analysis. Among the 10 biological variables
368 and enzyme activities, sequential tests indicated that the best DistLM ($R^2 = 0.68$; AICc =

369 122.4) was explained by 69.52% of total variation and identified four parameters
370 significant—BR, F/B-PLFA ratio, DHA and β-glucosidase—that were related to the soil
371 fungal community (Table S3 and Figure 1B). The dbRDA analysis clearly groups three
372 different clusters: cluster 1, composed of SS, Mix1 and Mix2 samples, was related to BR,
373 whereas cluster 2, consisting of CG and CC, was influenced by DHA; and finally,
374 reference soils (cluster 3) were influenced by F/B-PLFA ratio and β-glucosidase activity
375 (Figure 1B).

376 *3.5. Identification of fungal taxa by LEfSe analysis*

377 A linear discriminant analysis (LDA) effect size analysis (LEfSe) was performed to
378 identify the different fungal taxa with LDA scores of > 3.5 (Figure 2). These taxa showed
379 significant variation in their relative abundance in the soils studied (restored and natural
380 soils). LEfSe analysis revealed that, compared with the total fungal communities (57 at
381 genus level or the next available higher taxonomic level), 40 soil fungal taxa showed a
382 higher sensitivity to the conditions of the six soil types analysed (CG, CC, SS, Mix1,
383 Mix2 and NAT; Table S4). Specifically, LEfSe LDA results showed that CG-treated soils
384 favoured the presence of *Botryotrichum*, *uncultured* (Fam: Nectriaceae), *Mycosphaerella*,
385 *uncultured* (Fam: Chaetomiaceae), *Alfaria*, *Thermomyces* and *Neocamarosporium*,
386 whereas CC soils favoured those of *uncultured* (Fam: Microascaceae), *uncultured* (Fam:
387 Plectosphaerellaceae), *uncultured* (Fam: Spizellomycetaceae), *Iodophanus* and
388 *Stachybotrys* (Figure 2). Soils treated with sludge (SS) were associated with the genera
389 *Gymnascella*, *Lophotrichus* and *Chrysosporium*. Mixtures of sludge and compost showed
390 the genera *Cephaliophora* and *Arachniotus* in Mix1 (CG+SS), whereas in Mix2 (CC+SS)
391 the taxa *Microascus*, *Acremonium*, *uncultured* (Fam: Gymnoascaceae), Kernia and
392 *Arachnomyces* were present (Figure 2). The reference soils with the highest number of
393 differentiated fungal taxa (18 out of 40 total soil fungal taxa) with LDA > 4 were
394 *Exophiala, uncultured* (Class: Dothideomycetes), *Picoa*, *uncultured* (Order:
395 Chaetothyriales), *uncultured* (Order: Sebacinales), *uncultured* (Fam: Pyronemataceae)
396 and *uncultured* (Order: Pleosporales) with the Unclassified taxon having the highest LDA
397 (5.3) (Figure 2).

398 *3.6. Correlations between soil parameters and fungal populations*

399 Pearson's correlations of the 40 soil fungal taxa identified from LEfSe analysis with
400 physico-chemical soil parameters showed that taxa influenced by soils treated with SS

and Mix2 showed significantly ($p < 0.05$) positive correlations with the variables EC, TN and CH and significantly negative correlations with pH and C/N ratio (Figure 3A). Taxa associated with CC soils showed significantly positive correlations with pH and TOC and CH content. Those associated with CG and Mix1 soils scarcely presented significant correlations with the physico-chemical soil properties. On the contrary, the soil fungal community associated with reference soils showed significantly positive ($p < 0.05$) values with C/N ratio and positive values with soil pH, and significantly negative correlations with EC properties and TOC, TN, CH and POL content (Figure 3A).

Additionally, Pearson's correlations of these soil fungal taxa and biological soil properties (BR, PLFA, and enzymatic activity) were analysed. It was observed that the taxa identified for the CG-treated soils presented negative correlations with all the parameters analysed, except for the F/B-PLFA ratio, which were positive. Soils treated with CC showed positive correlations with DHA activity and F/B-PLFA ratio and negative with the rest of the soil parameters. In contrast, the soil fungal taxa from SS-treated soils indicated significantly positive correlations ($p < 0.05$) with BR, B-, S- and M-PLFA and negative correlations with the F/B-PLFA ratio and the three enzyme activities studied. Both Mix1 and Mix2 showed positive correlations with BR, being significantly ($p < 0.05$) positive in Mix2, and with the parameters of F-, B-, S-, M- and the S/M-PLFA ratio and negative with the F/B-PLFA ratio and the enzymatic activities (DHA, β-glucosidase and urease). Finally, fungal taxa from reference soils showed significantly positive correlations with F/B-PLFA ratio and β-glucosidase and urease activities and positive correlations with fungi and DHA activity but negative correlations for all other parameters.

3.7. Plant vegetation assessment

The principal species that colonised the experimental area were *Anthyllis cytisoides* L., *Pistacia lentiscus* L., *Capparis spinosa* L.*, Moricandia arvensis* L., *Atriplex halimus* L., *Limonium insigne* (Coss.) Kuntze, and *Artemisia barrelieri* Besser. Colonisation by wild plants increased considerably more over time in the restored plots than in the unrestored soils (CON; Figure S2). In C1, the percentage of total vegetation cover in the restored plots remained below 10%, except for the area with the CC-treatment, where the percentage of coverage was slightly higher. After 12 and 24 months of restoration (C2 and C3, respectively), the amended soils showed greater colonisation, with CG, CC and

433 the mixtures (Mix1 and Mix2) standing out, while SS presented the lowest values. On the
434 contrary, the CON soils presented similar values during the three campaigns (Figure S2).

435 **4. Discussion**

436 Our results demonstrated that the application of organic amendments improved the
437 physico-chemical, biochemical and biological soil properties and allowed the recovery of
438 plant cover in the first 18 months after the restoration. Several authors have indicated that
439 diversity and structure of the soil microbial community are driven by the physico-
440 chemical soil characteristics (Albornoz et al., 2016). Moreover, soil disturbances also
441 affect the soil microbial composition (Tian et al., 2017). Results showed that the soil
442 fungal communities were significantly different in the soils where several organic
443 amendments with distinct origins were applied (Table S2). Physico-chemical soil
444 properties (such as soil pH, salinity, TN content), biological parameters and enzymatic
445 activities (such as BR, F/B-PLFA ratio, and DHA and β-glucosidase enzymatic activities)
446 were the more important drives affecting soil fungal species (Figure 1).

447 Additionally, soil fungal communities were affected by the type of OM present in the
448 amendments applied. As was previously stated by Soria et al. (2020), both vegetable
449 composts (CG_0 and CC_0) applied in the restoration provided a more resilient OM,
450 especially CG, which in terms of fungal diversity and BR was closest to the reference
451 soils (Tables 2 and 3), suggesting the succession to a more mature fungal community
452 capable of mineralising more resilient C fractions (Paterson et al., 2008). Conversely, the
453 SS_0 treatment provided more labile OM with high TN and CH content (Rodríguez-Berbel
454 et al., 2021; Rocío Soria et al., 2021b), which benefited fast microbial growth (denoted
455 by BR, B- and F-PLFA and, β-glucosidase and urease activities; Table 2) in the sludge-
456 treated soils (SS, Mix1 and Mix2), causing a short-term PE (Soria et al., 2021).

457 However, the rapid mineralisation of labile C could be performed by the synergy among
458 soil fungal and bacterial communities, as indicated by the high content of B-PLFA and
459 the high BR (Table 2). Therefore, the chemical OM diversity provided by organic
460 amendments from different origin could favour on one hand, fungal communities
461 specialised in the use of specific substrates, and on the other hand, the excess multiple
462 types of nutrients, does not promote the need for specialisation by substrate
463 competitiveness (Allison et al., 2014; Nam et al., 2012).

464 The addition of organic amendments favoured the recovery of vegetation cover in the
465 restored soils resulting in a greater proliferation of plants than in the control soils, which
466 were practically devoid of them (Figure S2). Plants could have also favoured the
467 proliferation and diversity of the fungal communities in the restored soils (Figure S1). For
468 example, this proliferation could been due to the contribution of photosynthetic
469 assimilates from vegetation to rhizospheric microorganisms (Frouz, 2021), leading to a
470 renewal of soil biodiversity and the establishment of ecosystem functionality (Conesa et
471 al., 2012).

472 Results of fungal diversity in this soils corroborated that the organic amendments allowed
473 the specialisation and establishment of different soil fungal communities depending on
474 the organic substrates provided to the restored soils (Soria et al., 2020) and those inherited
475 from amendments. An example of the latter could be the incorporation into soils of fungi
476 of the phylum Ascomycota. Many species of this phylum are involved in the
477 decomposition of organic complex substrates (Guo et al., 2018; Liu et al., 2019) of plant
478 biomass as well as in C and N cycles in arid ecosystems (Challacombe et al., 2019). This
479 results in the increase of available recourses which can favour the emergence of other
480 phyla, causing soil fungal taxa diversification, which can occupy more niches and reduce
481 competition (Xiong et al., 2021).

482 The idea that OM decomposition from plants is taking place in these soils is reinforced,
483 especially in CC-treated and reference soils, by the presence of the saprophytic phylum
484 Basidiomycota (Curlevski et al., 2010), capable of metabolising plant inputs rich in lignin
485 (Blackwood et al., 2007). Indeed, CC-treated soils showed high values of TOC and POL,
486 probably from decomposition of more resilient OM (Soria et al., 2020). Fungi can secrete
487 hydrolytic enzymes capable of decomposing complex CH (Berlemont, 2017; Kabel et al.,
488 2020), mineralising OM (Fontaine et al., 2003), and releasing nutrients (Hellequin et al.,
489 2018), that can be used for the rest of microorganisms and for plants. The presence of
490 more mature fungal taxa capable of degrading more resilient compounds is corroborated
491 by the fact that after 18 months the restored soils showed values of biological soil
492 properties such as BR, B-PLFA, F/B-PLFA ratio and DHA activity that were closer to
493 those of the reference soils than to those described 6 months after the restoration by
494 Rodríguez-Berbel et al. (2021).

495 In the restored plots, we found fungal taxa probably inherited by the initial organic
496 amendments (CG_0, SS_0 and CC_0) and others common to the reference soils. For example,
497 taxa such as *uncultured* (Fam: Plectosphaerellaceae) and *uncultured* (Fam:
498 Chaetomiaceae) could be incorporated in soils from vegetable composts (CG and/or CC),
499 whereas other taxa such as *Cephaliophora*, *Acremonium*, *Gymnascella* and *uncultured*
500 (Fam: Microascaceae) were already present in reference soils (Table S2).

501 Statistical LEfSe analysis denoted indicator taxa for each treatment (Figure 2). The fungal
502 taxon *uncultured* (Fam: Microascaceae) associated with the CC-treated soils could be
503 indicative of an advanced stage of soil maturity because it has been observed in maturing
504 stages of composting (Jiang et al., 2020; Klamer and Bååth, 2006). Another example of a
505 CC-indicator was the genus *Stachybotrys,* described in arid soils (Zak and Wildman,
506 2004) and capable of producing cellulolytic enzymes as β-glucosidases (Amouri and
507 Gargouri, 2006).

508 In relation to CG-taxa indicators, the Chaetomiaceae family was previously described as
509 one of the main taxonomic groups that can help to differ untreated soils from organically
510 treated soils (Banerjee et al., 2019). Thus, their presence in CG soils could indicate
511 complex metabolisation of OM, especially in cellulose degradation (Paula et al., 2020).
512 Interestingly, another CG-indicator was the genus *Neocamarosporium* (Figure 2),
513 described as a leaf endophyte (Ricks and Koide, 2019) of halophytic plants (Gonçalves
514 et al., 2019). Its presence could indicate that the spontaneous vegetation established in
515 these plots (Figure S2) has favoured the fungal diversity of restored soils.

516 According to SS-indicator soil taxa, the genus *Gymnascella* was identified with
517 significantly positive correlations with EC and TN (Figure 3a). This genus has species
518 such as *G. dankaliensis* y *G. hyalospora* that have been identified as naturally inhabiting
519 manure and soils (Abdel-Azeem et al., 2011), including saline and desert soils (Sybren
520 de Hoog et al., 2005). Other species of this genus present an important biotechnological
521 potential for obtaining pharmaceuticals (Nicoletti and Andolfi, 2018).

522 In soils where mixes of organic amendments were applied, the genus *Cephaliophora*,
523 which has been described as a decomposer of cellulose (Asemaninejad et al., 2021), can
524 be highlighted as a Mix1-indicator, whereas as a Mix2-indicator the genus *Acremonium*
525 was described as decomposer and degrader of cellulose and xylan (Sun et al., 2016) and
526 had significantly positive correlations with BR and CH (Figure 3b). Another Mix2-

527 indicator taxa, the genus *Microascus,* was isolated from soils and decomposing plant
528 material (Piñar et al., 2019). The increase in the relative abundance of fungal taxa
529 indicators of mixed soils (Mix1 and Mix2) with respect to SS-treated soils (Table S2)
530 could suggest that the combination of labile and recalcitrant OM from organic
531 amendments would have benefited the development of these taxa.

532 Lastly, the natural soils used as reference showed the highest number of indicators fungal
533 taxa (Figure 2). This could be explained because these ecosystems are balanced and taxa
534 are in a steady state with a well-structured soil microbial community because soil fungi
535 have the ability to adapt better to nutrient-poor soils by searching for heterogeneously
536 distributed resources (Boer et al., 2005). Among NAT-indicators taxa, we found
537 endophytic fungi such as order Botryosphaeriales and genus *Exophiala* (Singh et al.,
538 2017; Zak and Wildman, 2004) or lichen-forming fungi such as genus *Verrucaria* (Sybren
539 de Hoog et al., 2005). Another NAT-indicator taxon, class Agaricomycetes, characterised
540 as saprotrophic and mycorrhizal fungi (Collins et al., 2018; Hart et al., 2019) was
541 commonly described in semiarid soils (Bastida et al., 2013; Tian et al., 2017).

542 Previous results showed that the fungal communities of the restored soils could be playing
543 important functions, facilitating the biogeochemical cycles in these soils compared with
544 the unrestored soils. In addition, it was also observed that the restoration favoured the
545 transformation of OM. Interestingly, Soria et al. (2021b) noted that C losses to the
546 atmosphere were low and that the experimental plots could act as CO_2 sinks.

547 5. Conclusions

548 Medium-term (18 months) additions of organic amendments influenced soil physico-
549 chemical, biological and microbial variables, generating changes in soil microbial
550 activities and nutrient use efficiency. These changes were correlated with a change in
551 fungal community composition, which was driven principally by the chemical diversity
552 that composes the OM. Organic amendments served mainly to support microbial growth
553 in restored soils (e.g., *Stachybotrys* and *Acremonium* genera), which are associated with
554 the decomposition of complex OM and soil nutrient transformations, and then enhanced
555 soil fertility for soils degraded by mining activity. The presence of fungi in the restored
556 plots, some common in natural soils, suggests that ecological succession has occurred in
557 these soils approaching the quality status of natural soils in the environment, whereas in
558 the control soils fungi have not proliferated despite the time elapsed since degradation,

suggesting that these soils alone have not been able to recover in the medium term from the impact of mining. These results corroborate that restoration treatments were useful for the recovery of soil biological quality, although the lower fungal diversity in restored versus natural soils also suggests that the status of natural soils 18 months after restoration has not been achieved. However, the inherent mechanisms require better understanding through future research aimed at determining a direct link between enzymatic activities and the responsible fungal taxa in soils, and metatranscriptome/metaproteome analyses should provide promising ways to obtain direct proof of the claims presented in this study.

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Tables

Table 1. Physico-chemical soil properties of restored and undisturbed natural soils (mean ± SEM [n = 3]). Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PERANOVA).

	CON	**CG**	**Mix1**	**SS**	**Mix2**	**CC**	**NAT**
pH	8.67 ± 0.06 b	7.99 ± 0.09 a	8.07 ± 0.09 a	7.88 ± 0.06 a	7.97 ± 0.08 a	8.87 ± 0.16 b	8.70 ± 0.00 b
EC (mS/cm)	1.64 ± 0.35 b	2.23 ± 0.43 ab	2.75 ± 0.68 ab	3.24 ± 0.04 a	2.6 ± 0.42 ab	1.75 ± 0.35 b	0.08 ± 0.00 c
TOC (%)	0.81 ± 0.16 d	2.65 ± 0.03 a	2.46 ± 0.39 abc	3.08 ± 0.20 ab	2.90 ± 0.21 ab	3.37 ± 0.17 b	1.81 ± 0.14 c
TN (%)	0.06 ± 0.00 c	0.38 ± 0.00 a	0.42 ± 0.04 ab	0.52 ± 0.02 b	0.42 ± 0.05 ab	0.40 ± 0.01 a	0.15 ± 0.01 d
C/N ratio	12.8 ± 2.62 cd	6.97 ± 0.17 a	5.76 ± 0.69 ab	5.90 ± 0.25 b	6.87 ± 0.29 ab	8.40 ± 0.22 c	11.6 ± 0.43 d
CH ($\mu g\ g^{-1}$)	0 ± 0 c	470.68 ± 90.7 a	566.20 ± 248. ab	770.13 ± 93.2 a	338.75 ± 115. ab	156.74 ± 20.8 b	38.12 ± 4.75 d
POL ($\mu g\ g^{-1}$)	0 ± 0 d	20.05 ± 0.74 ac	24.42 ± 5.74 ab	26.36 ± 8.80 abc	26.98 ± 4.71 ab	35.78 ± 5.32 b	6.198 ± 3.25 c

CON: unrestored soils; CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils. EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; C/N ratio: carbon to nitrogen ratio; CH: carbohydrates content; POL: polyphenols content.

Table 2. Soil basal respiration, fatty acids profile and enzyme activities in restored soils with organic amendments and natural reference soils (average ± SEM [n = 3]). Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PERANOVA).

	CON	CG	Mix1	SS	Mix2	CC	NAT
BR (mg C-CO2 kg^{-1} soil day)	0.57 ± 0.33 a	3.61 ± 2.08 b	12.0 ± 6.95 d	17.0 ± 9.82 d	13.1 ± 7.59 d	5.93 ± 3.42 c	3.71 ± 2.14 bc
Fatty acids profile							
F-PLFA (nmol g^{-1} soil)	2.10 ± 1.21 a	10.30 ± 5.95 b	40.52 ± 23.39 c	44.48 ± 25.68 c	30.27 ± 17.47 c	27.16 ± 15.68 c	41.10 ± 23.73 c
B-PLFA (nmol g^{-1} soil)	19.14 ± 11.05 a	106.61 ± 61.55 b	340.77 ± 196.74 cd	466.54 ± 269.36 d	265.27 ± 153.15 c	175.04 ± 101.06 c	201.28 ± 116.21 c
F/B-PLFA ratio (nmol g^{-1} soil)	0.11 ± 0.06 abc	0.09 ± 0.05 ab	0.11 ± 0.06 ac	0.09 ± 0.05 b	0.11 ± 0.06 abc	0.15 ± 0.08 abc	0.20 ± 0.11 c
S-PLFA (nmol g^{-1} soil)	17.40 ± 10.05 a	120.53 ± 69.58 b	339.23 ± 195.85 cd	552.87 ± 319.20 c	272.80 ± 157.50 d	158.62 ± 91.58 b	193.16 ± 111.52 d
M-PLFA (nmol g^{-1} soil)	10.64 ± 6.14 a	54.80 ± 31.64 b	166.53 ± 96.15 bcde	206.3 ± 119.10 c	130.2 ± 75.17 de	101.6 ± 58.69 d	106.7 ± 61.65 e
S/M-PLFA ratio (nmol g^{-1} soil)	1.64 ± 0.94 a	2.18 ± 1.26 a	2.24 ± 1.29 a	2.67 ± 1.54 a	2.12 ± 1.22 a	1.68 ± 0.97 a	1.83 ± 1.06 a
Enzyme activities							
DHA (µmol INTF g^{-1} soil h^{-1})	0.04 ± 0.02 e	0.22 ± 0.13 a	0.34 ± 0.20 ad	0.65 ± 0.37 bc	0.52 ± 0.30 cd	0.94 ± 0.54 bc	0.87 ± 0.50 b
β-glucosidase (µmol PNF g^{-1} soil h^{-1})	0.02 ± 0.01 c	0.15 ± 0.08 a	0.23 ± 0.13 ab	0.35 ± 0.20 b	0.32 ± 0.18 b	0.28 ± 0.16 b	0.60 ± 0.35 d
Urease (µmol N-NH_4^+ g^{-1} soil h^{-1})	0.04 ± 0.02 a	0.24 ± 0.14 c	0.25 ± 0.14 ac	0.52 ± 0.30 abc	0.36 ± 0.21 c	0.45 ± 0.26 abc	1.34 ± 0.77 b

CON: unrestored soils; CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils. BR: basal soil respiration; F-PLFA: fatty acids of fungal biomass; B-PLFA: fatty acids of bacterial biomass; F/B ratio: ratio between fungus and bacteria fatty acids; S-PLFA: saturated fatty acids; M-PLFA: monosaturated fatty acids; S/M-PLFA ratio: ratio between saturated and monounsaturated fatty acids; DHA: dehydrogenase activity.

Table 3. Alpha-diversity analysis (average ± SEM; n = 3) of ASVs richness, Pielou, and Shannon indices of different soil types. Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PERANOVA).

	CON	CG	Mix1	SS	Mix2	CC	NAT
ASVs	-	120 ± 8.95 a	81 ± 6.38 cd	75 ± 15.89 bc	83 ± 6.08 c	80 ± 12.99 bd	157 ± 7.96 e
Pielou	-	0.72 ± 0.03 abc	0.68 ± 0.02 ce	0.63 ± 0.02 d	0.63 ± 0.01 e	0.68 ± 0.02 b	0.76 ± 0.03 a
Shannon	-	5.00 ± 0.29 abc	4.32 ± 0.05 c	3.92 ± 0.32 bc	4.03 ± 0.13 b	4.31 ± 0.16 cd	5.55 ± 0.18 ad

CON: unrestored soils (no data obtained); CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.

Supplementary table 1. Number of sequences per sample (n = 21) considered in alpha-diversity estimate and utilised for assignment of taxonomy from QIIME2 software.

	Sample ID	Feature count	Average ± SEM
CON	P6	0	-
	P12	2639	
	P18	0	
CG	P1	27373	54115 ± 16754
	P7	84972	
	P14	50002	
Mix1	P4	63081	66533 ± 3160
	P10	63675	
	P16	72845	
SS	P2	101859	100342 ± 5868
	P8	89505	
	P15	109664	
Mix2	P5	99628	87825 ± 10328
	P11	67242	
	P17	96607	
CC	P3	29982	56585 ± 14411
	P9	60281	
	P13	79494	
NAT	N1	58628	69447 ± 20831
	N2	40015	
	N3	109700	
Total Feature count		1304553	72475 ± 6098

CON: unrestored soils (these samples were removed from the analysis because they did not satisfy the sequence quality filters); CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.

Supplementary table 2. Relative abundance percentage of soil fungal taxa in organic amendments to restored and natural soils. The 13 of the 57 most abundant genera or the next available higher taxonomic level are shown in the legend (relative abundance > 0.1%). Different letters indicate statistical differences for each treatment ($p < 0.05$; one-way PERMANOVA).

	Amendments			Restored soils					
	CG_0	**SS_0**	**CC_0**	**CG**	**Mix1**	**SS**	**Mix2**	**CC**	**NAT**
Unclassified	0	0	0	0.2	0.0	0.0	0.1	0.2	1.2
Microascus	0.8	2.3	0.3	0.0	0.6	0.7	0.8	0.0	0.0
uncultured (Fam: Microascaceae)	0	0	0	0.2	0.1	0.1	0.4	0.9	0.0
Cephaliophora	0	0	0	0.2	1.0	0.3	0.2	0.0	0.0
Acremonium	0	0	0	0.0	0.4	0.3	0.4	0.1	0.0
uncultured (Fam: Nectriaceae)	0	0	0	0.5	0.2	0.1	0.1	0.3	0.0
Botryotrichum	0	0	0	0.6	0.4	0.1	0.1	0.1	0.0
uncultured (Fam: Plectosphaerellaceae)	0.2	0	2.5	0.0	0.0	0.0	0.1	0.5	0
Gymnascella	0	0	0	0.0	0.1	0.6	0.3	0.0	0.0
Exophiala	0	0	0	0	0	0	0.0	0	0.3
Alternaria	0	0	0	0.5	0.1	0.2	0.1	0.2	0.0
Lophotrichus	0	0	0	0.0	0.1	0.3	0.2	0.0	0.0
uncultured (Class: Dothideomycetes)	0	0	0	0.0	0	0	0	0	0.2
Sodiomyces	0.2	0	0	0.1	0.0	0.0	0.0	0.1	0.0
uncultured (Fam: Chaetomiaceae)	0	0	0.9	0	0	0	0	0	0
uncultured (Class: Eurotiomycetes)	1.3	1.0	1.0	0	0	0	0	0	0
uncultured (Order: Eurotiales)	0.4	0.8	0.7	0	0	0	0	0	0
Phialosimplex	1.1	0	0	0	0	0	0	0	0
				a	**b**	**c**	**b**	**d**	**e**

CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.

Supplementary table 3. Distance-based redundancy analysis results, explanatory variance and contribution of relative abundance of soil fungal taxa. physico-chemical and biological soil characteristics.

Relative abundance of soil fungal taxa and physico-chemical soil properties

Percentage of variation explained by individual axes (% explained variation out of total variation)

Axis	Individual
1	47.57
2	12.41

Weights

(Coefficients for linear combinations of X's in the formation of dbRDA coordinates)

Variable	dbRDA1	dbRDA2
Soil pH	-2.11	18.90
Electrical conductivity (EC)	15.33	10.81
Total nitrogen (TN)	11.51	2.57

Relative abundance of soil fungal taxa and biological soil properties

Percentage of variation explained by individual axes (% explained variation out of total variation)

Axis	Individual
1	51.05
2	18.47

Weights

(Coefficients for linear combinations of X's in the formation of dbRDA coordinates)

Variable	dbRDA1	dbRDA2
β-glucosidase	-15.51	21.76
Basal respiration (BR)	19.65	8.10
F/B-PLFA ratio	-5.28	-2.99
Dehydrogenase (DHA)	1.45	-12.73

Supplementary table 4. LDA bars indicate the fungal soil communities within the experimental plots with an LDA score of > 3.5.

Selected Taxa	LDA Score	Associated Group
7: *Botryotrichum*	4.93	CG
6: [F] Nectriaceae	4.85	CG
14: *Mycosphaerella*	4.36	CG
19: [F] Chaetomiaceae	4.34	CG
54: *Alfaria*	4.27	CG
56: *Thermomyces*	4.22	CG
18: *Neocamarosporium*	4.18	CG
4: *Cephaliophora*	5.13	Mix1
48: *Arachniotus*	3.56	Mix1
9: *Gymnascella*	4.92	SS
12: *Lophotrichus*	4.69	SS
31: *Chrysosporium*	4.19	SS
2: *Microascus*	5.04	Mix2
5: *Acremonium*	4.78	Mix2
22: *Gymnoascaceae unidentified*	4.04	Mix2
36: *Kernia*	3.83	Mix2
29: *Arachnomyces*	3.82	Mix2
3: [F] Microascaceae	5.03	CC
8: [F] Plectosphaerellaceae	4.77	CC
34: *Spizellomycetaceae unidentified*	4.05	CC
49: *Iodophanus*	4.00	CC
41: *Stachybotrys*	3.90	CC
1: Unclassified	5.28	NAT
10: *Exophiala*	4.79	NAT
13: [O] Dothideomycetes	4.54	NAT
15: *Picoa*	4.44	NAT
17: *Chaetothyriales unidentified*	4.34	NAT
21: *Sebacinales unidentified*	4.24	NAT
20: *Pyronemataceae unidentified*	4.17	NAT
24: *Pleosporales unidentified*	4.10	NAT
23: [O] Pleosporales	4.05	NAT
35: [F] Didymosphaeriaceae	3.98	NAT
38: *Darksidea*	3.97	NAT
30: *Mortierella*	3.95	NAT
33: [P] Ascomycota	3.92	NAT
40: *Verrucaria*	3.89	NAT
39: *Knufia*	3.89	NAT
45: [C] Agaricomycetes	3.87	NAT
46: *Clitopilus*	3.84	NAT
51: *Botryosphaeriales unidentified*	3.70	NAT

CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils. [C]: Fungal soil taxon identified to class level; [O]: Fungal soil taxon identified to order level; [F]: Fungal soil taxon identified to family level.

__Figures__

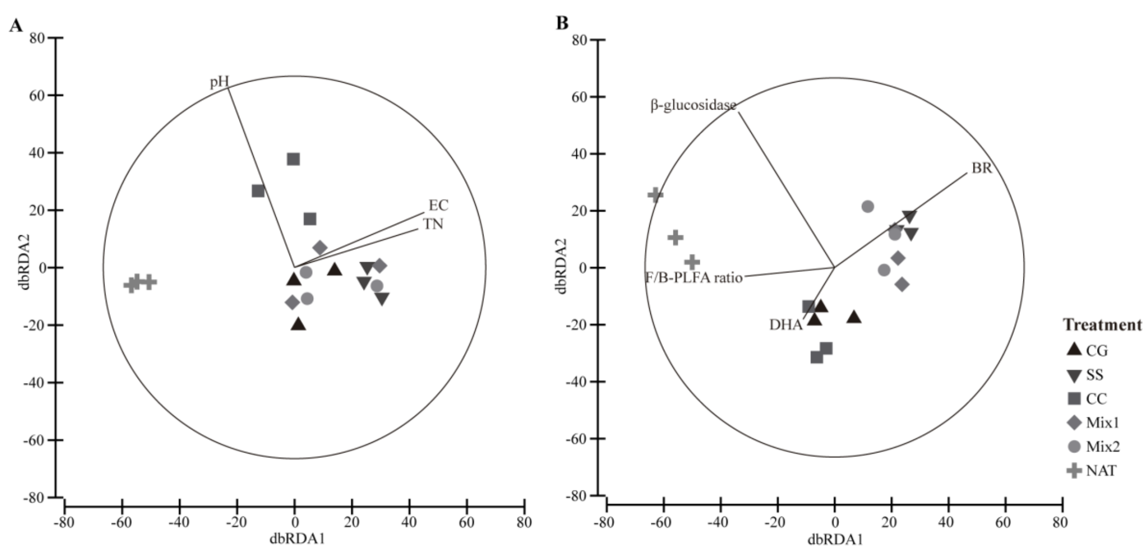

Figure 1. Redundancy analysis (dbRDA) showing the correlation among physico-chemical (A) and microbiological (B) soil properties (basal soil respiration, fatty acids profile and enzyme activities) and fungal soil community based on the relative abundance (%) of richness ASVs. Soil samples with compost from garden waste (CG), sewage sludge from wastewater (SS), compost from greenhouse crop residues (CC), Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge and natural reference soils (NAT) are indicated by different colours and figures.

Footnotes: Soil characteristics are represented by continuous lines. EC: electrical conductivity; TN: total nitrogen; BR: soil basal respiration; F/B-PLFA ratio: ratio between fungi and bacteria fatty acids; DHA: dehydrogenase activity.

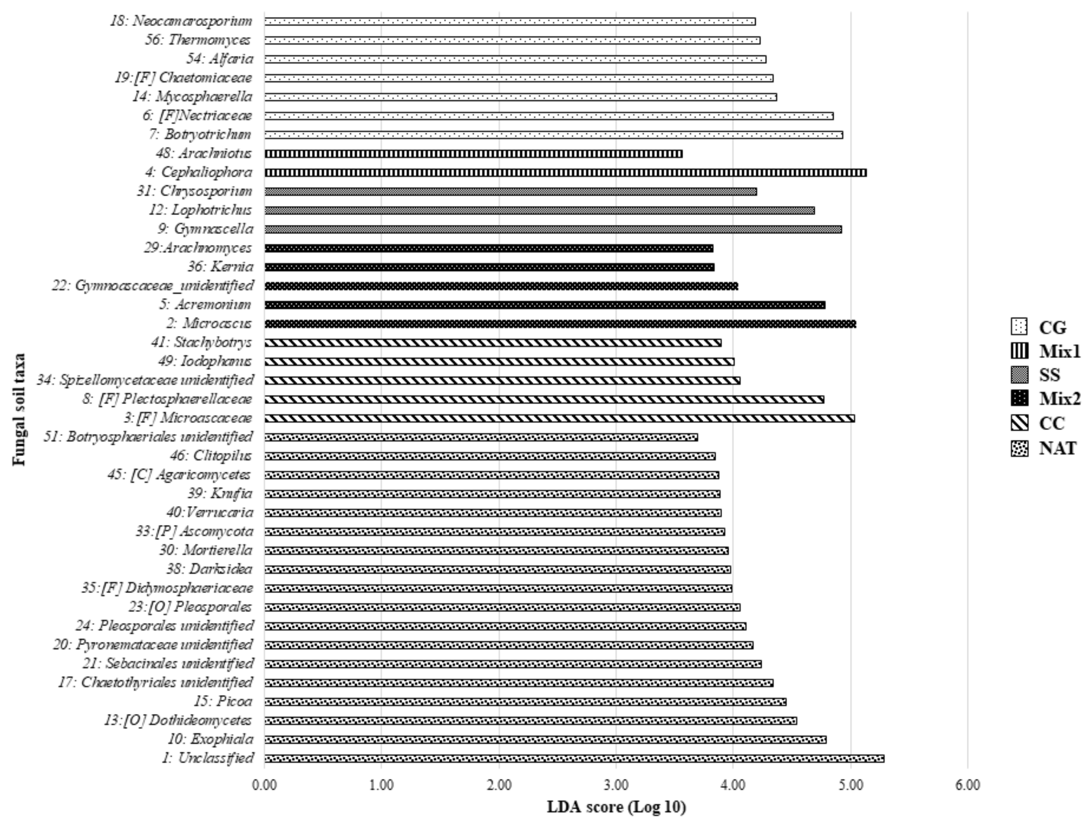

Figure 2. Liner discriminant analysis coupled with effect size (LEfSe) measures between different treatments (both composts, CG and CC; sewage sludge, SS; and mixtures, Mix1 and Mix2, among previous) and natural reference soils (NAT). Lineages with LDA values greater than 3.5 are shown.

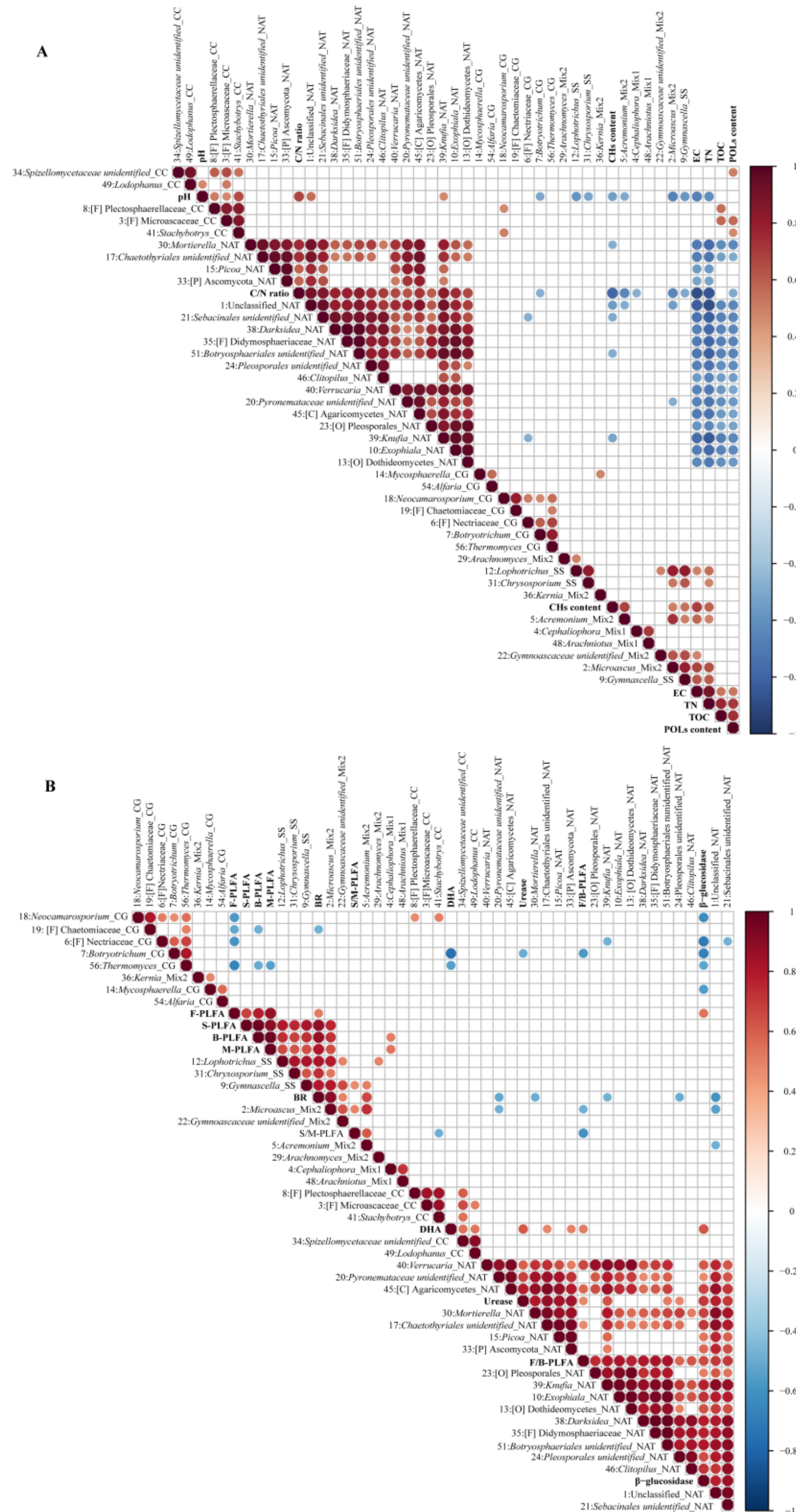

Figure 3. Pearson correlation ($p < 0.05$) between physico-chemical (A) and microbiological soil characteristics (basal respiration, fatty acids and enzyme activities) (B) with 40 soil fungal taxa from LEfSe analysis.

Footnotes: The different soil properties analysed are shown in bold. [C]: Fungal soil taxon identified to class level; [O]: Fungal soil taxon identified to order level; [F]: Fungal soil taxon identified to family level.

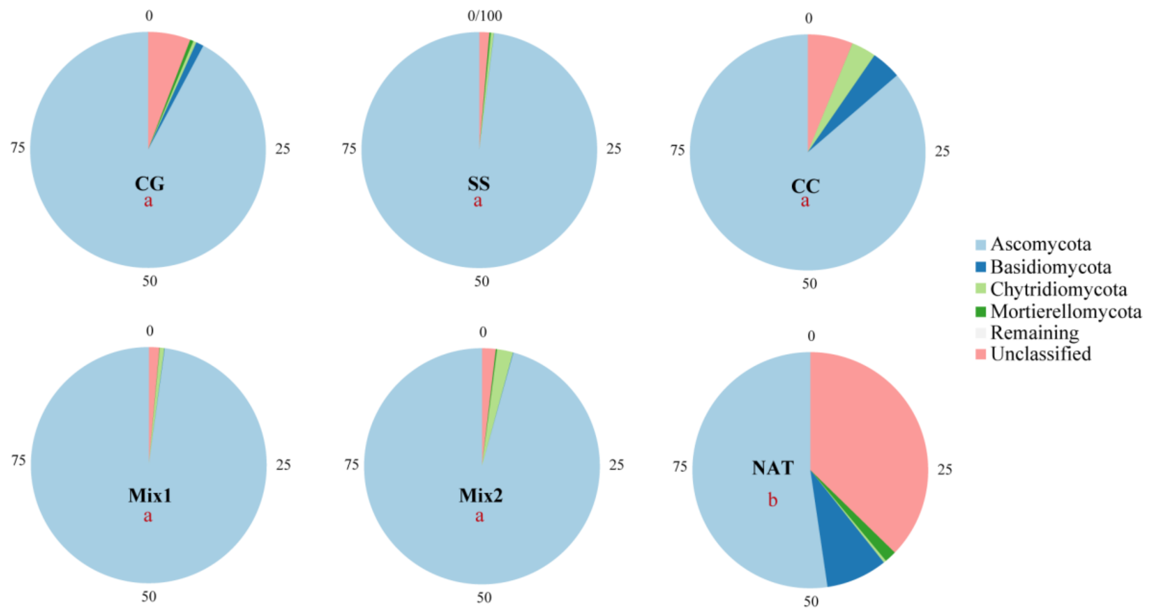

Figure S1. Pie charts based on the relative abundance (%) of soil fungal phyla. Every pie shows the percent of the relative abundance (%) of the fungal phyla of different treatments applied. Different red letters indicate statistical differences for each treatment ($p < 0.05$; PERMANOVA).

Footnotes: CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils

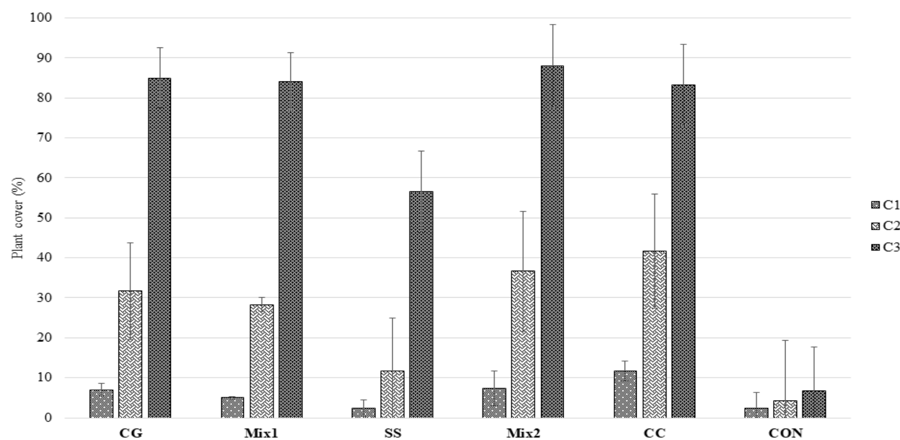

Figure S2. Percent of the total vegetation cover on experimental plots after 6 (C1), 12 (C2) and 24 months (C3) of restoration.

Footnotes: CG: compost from garden waste; CC: compost from greenhouse crop residues: SS: sewage sludge from wastewater; Mix1 (CG + SS) and Mix2 (CC + SS): mixtures of amendments from different vegetal compost and sewage sludge; NAT: natural reference soils.