Patterns of change in α and β taxonomic and phylogenetic diversity in the secondary succession of semi-natural grasslands in the Northern Apennines

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We studied the secondary succession in semi-natural grasslands (dry grasslands and hay meadows) located in the eastern side of the Tuscan Apennines (Tuscany, Central Italy). We compared these habitats, investigating: i) the changes in species richness, composition and phylogenetic diversity during the succession; ii) whether the trends in species loss and species turnover in taxonomic diversity matched those in phylogenetic diversity. We performed a stratified random sampling, in a full factorial design between habitat type and succession stage (60 sampled plots, 10 x 2 types of habitat x 3 stages of succession). We constructed a phylogenetic tree of the plant communities and compared the differences in taxonomic/phylogenetic α- and β-diversity between these two habitats and during their succession. We identified indicator species for each succession stage and habitat. Looking at α-diversity, both habitats displayed a decrease in species richness, with a random process of species selection in the earlier succession stages from the species regional pool. Nevertheless, in the latter stage of dry grasslands we recorded a shift towards phylogenetic overdispersion at the higher-level groups in the phylogenetic tree. In both habitats, while the richness decreased with succession stage, most species were replaced during the succession. However, the hay meadows were characterized by a higher rate of new species' ingression whereas the dry grasslands became dominated with *J. communis*. Accordingly, the two habitats showed similar features in phylogenetic β-diversity. True phylogenetic turnover resulted the main component, due to replacement of unique lineages along the succession. Nevertheless, in dry grasslands this trend is slightly higher than expected considering the major importance of difference in species richness of dry grasslands sites, and this is due to the presence of a phylogenetically very distant species (*J. communis*).

1 **Patterns of change in α and β taxonomic and phylogenetic diversity in the secondary**
2 **succession of semi-natural grasslands in the Northern Apennines**

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Abstract

18 We studied the secondary succession in semi-natural grasslands (dry grasslands and hay
19 meadows) located in the eastern side of the Tuscan Apennines (Tuscany, Central Italy). We
20 compared these habitats, investigating: i) the changes in species richness, composition and
21 phylogenetic diversity during the succession; ii) whether the trends in species loss and species
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25 phylogenetic tree of the plant communities and compared the differences in
26 taxonomic/phylogenetic α- and β-diversity between these two habitats and during their
27 succession. We identified indicator species for each succession stage and habitat. Looking at α-
28 diversity, both habitats displayed a decrease in species richness, with a random process of
29 species selection in the earlier succession stages from the species regional pool. Nevertheless, in
30 the latter stage of dry grasslands we recorded a shift towards phylogenetic overdispersion at the
31 higher-level groups in the phylogenetic tree. In both habitats, while the richness decreased with
32 succession stage, most species were replaced during the succession. However, the hay meadows
33 were characterized by a higher rate of new species' ingression whereas the dry grasslands
34 became dominated with *J. communis*. Accordingly, the two habitats showed similar features in
35 phylogenetic β-diversity. True phylogenetic turnover resulted the main component, due to
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39 (*J. communis)*.
40

Introduction

Secondary semi-natural grasslands represent important components of European cultural landscapes. They derive from centuries of traditional land use, mainly linked to grazing by livestock (pastures) or hay-making (meadows) (Dengler *et al.* 2014; Janišová *et al.* 2011). Many secondary grassland vegetation types are considered habitats worthy of conservation and are listed in European and national protection directives and laws, such as the Low altitude hay meadows and the *Festuco-Brometea* dry grasslands (respectively codes 6510 and 6210, according to European Council Directive 92/43/EEC). In Europe, because of recent cultural changes, secondary grasslands and meadows have displayed an overall tendency to evolve into shrublands and woodlands through natural secondary successions (Dengler *et al.* 2014; Monteiro *et al.* 2011; Peco *et al.* 2006; Peco *et al.* 2012). Perennial grasslands undergo a vegetation dynamism leading to the gradual transition from herbaceous coenoses belonging to syntaxonomical classes such as *Festuco-Brometea* or *Molinio-Arrhenatheretea* (e.g. Allegrezza and Biondi, 2011; Biondi *et al.* 1995) to shrub coenoses belonging, especially in central Italy, mainly to the *Rhamno-Prunetea* class (e.g. Biondi *et al.* 1988). The shrubland formations are sometimes preceded by intermediate phases dominated by other herbaceous species such as *Brachypodium rupestre* (Assini *et al.* 2014) and mostly originate from the species that form the fringes of woods surrounding grasslands (Biondi *et al.* 1988; Poldini *et al.* 2002).

Such succession, with the consequent loss in species, has been widely studied from a landscape viewpoint (Bracchetti *et al.* 2012; Rocchini *et al.* 2006; Viciani *et al.* 2018). Many authors have also focused on the functionality of the communities, elucidating the central role of competition in the loss of species characterizing the early phases of succession (Csergő *et al.* 2013; Lepš 1999; Peco *et al.* 2012). Nevertheless, less is known regarding the changes occurring in the communities with respect to trends in β-diversity phylogenetic relationships, also concerning their link with changes at the taxonomic level. Indeed, according to Chase and Myers (2011), β-diversity can provide considerable insights into the importance of deterministic and stochastic processes in generating community structure along spatial and ecological gradients. In addition, the use of molecular phylogenies may be helpful in analyzing the forces that influence patterns of biodiversity and biogeography, and in depicting the interactions among co-occurring species (Selvi *et al.* 2016). Indeed, in the last decades, the use of molecular phylogeny has increased widely for ecological purposes, contributing also to the emerging area of community phylogenetics (Webb *et al.* 2002). One of the multiple ways to use the phylogenetic information consists in the measure of the phylogenetic overdispersion or clustering of the community in relation to the variation of the habitat conditions (Erickson *et al.* 2014; Qian *et al.* 2014; Selvi *et al.* 2016). Recently, many authors (Webb 2000; Webb *et al.* 2002; Kembel 2009) have highlighted that the observed patterns of phylogenetic structure of the communities could be used to understand the processes of community assembly, particularly linking patterns of phylogenetic clustering and overdispersion with the processes of habitat filtering and competitive exclusion. Indeed, the use of phylogenetic patterns as proxies for the processes of community assembly is rapidly raising concerns linked to the assumptions underlying such approach, often

81   only weakly supported. In particular, these assumptions regard the existence of an actual

82   correlation between measures of phylogenetic dispersion and trait dispersion, the idea that trait

83   similarity would enhance competition and that competition necessarily causes species exclusion

84   from the community, and that community assemblages are in status of equilibrium (see Gerhold

85   *et al.* 2015; Prinzing 2016). Furthermore, Kraft *et al.* (2015) reported a misuse of the concept of

86   environmental filtering, considering that most empirical studies hardly distinguish the effects of

87   abiotic factors from those of biotic interactions, and often overestimate the role of the

88   environment in shaping communities.

89   In our study area, the Tuscan Apennines, the co-occurrence of initial species in secondary

90   grasslands is driven by different agricultural management in different geo-morphological

91   conditions. The areas with low slope inclination and relatively fertile and deep soils are at first

92   subjected to machining and sowing, mainly with plants that increase the nutrient value in the soil

93   (i.e. *Medicago sativa, Onobrychis viciifolia*). Then, herbaceous natural grassland species (many

94   from the families *Poaceae, Fabaceae* and *Asteraceae*) start to colonize these communities,

95   which in a few years become semi-natural hay meadows (Ubaldi 2003). On the other hand, areas

96   on steeper slopes with shallow (sometime even rocky) soils are used as pastures and become dry

97   grasslands. Also in our area of study, the main characteristic differentiating the two habitat types

98   is the stand geomorphology, with significantly steep dry grasslands and almost flat hay

99   meadows: these differences may affect the water capacity, structure and fertility of soils.

100   We expect that these ecological differences may also affect the secondary succession, leading to

101   different species assemblages. Indeed, as anthropic pressures are relieved, the succession of these

102   habitats is generally characterized by intermediate phases dominated by herbaceous species,

103   leading finally to quite different shrub formations. In particular, while shrubland following hay

104   meadows are dominated by several species of broadleaf shrubs, dry grasslands are dominated by

105   *Juniperus communis*, a distantly related stress-tolerant species (Pierce *et al.* 2017).

106   The study of taxonomic, phylogenetic and functional plant (α and β) diversity along secondary

107   succession has already been the object of studies (see Purscke *et al.* 2018), which highlighted

108   that the relative importance of assembly processes had changed over time, but with contrasting

109   patterns of temporal change in the different facets of diversity. Nevertheless, while Purscke *et al.*

110   (2013) observed a general increase in taxonomic, phylogenetic and functional alpha-diversity

111   during succession, we expect a decrease in species taxonomic diversity, not necessarily linked to

112   a decrease in phylogenetic diversity. Moreover, Purscke *et al.* (2013) reported a predominant role

113   of abiotic filtering in community assembly during the early stages of grassland succession,

114   whereas the relative importance of competitive exclusion appears to have increased towards the

115   later succession stage. Conversely, a major role of competition by dominant tall grasses as

116   already been described for the early stages of succession in Apennine grasslands communities

117   (Corazza *et al.,* 2016). According to these differences, we sought to i) assess the relative changes

118   in α- and β-diversity taxonomic and phylogenetic diversity, also identifying the indicator species

119   in the different stages of the succession and ii) assess whether the trends in species loss and

120   species turnover in taxonomic diversity matches those in phylogenetic diversity. Moreover, we

121 further hypothesize that *Juniperus communis* may have a pivotal role, driving a certain degree of
122 overdispersion of the phylogenetic structure of the communities in dry grasslands, considering its
123 distant relatedness.
124 Toward these aims, we conducted a sampling of dry grasslands and hay meadows plant
125 communities in the Tuscan Apennines, adopting a chrono-sequential approach to reconstruct
126 their dynamic changes, assessing the changes in of α- and β-diversity within and between three
127 succession stages of these two habitats.
128
Materials & Methods
130
Study area
132 The study area is located in the Adriatic side of the south-eastern Tuscan Apennines (Tuscany,
133 Central Italy, 43.691838°N 12.111936°E). The area is managed by the Raggruppamento
134 Carabinieri Biodiversità of the Reparto Carabinieri Biodiversità Pieve Santo Stefano. The most
135 represented geological units are the clays of the Sillano Formation, deposits of landslide debris
136 and, to a lesser extent, the sandstones of Falterona Mount (Carmignani *et al.* 2013). Climate
137 belongs to the Temperate Oceanic Bioclimate (Pesaresi *et al.* 2017).
138
Sampling design
140 The study was carried out exploiting a random/stratified sampling design involving two layers:
141 type of habitat (hereafter Habitat) and stage of succession (hereafter Succession). To identify and
142 map these layers correctly, we performed an analysis of orthophotos of the study area using the
143 QGis software (version 2.14.21, QGIS.ORG project). We used the cover percentage of scrub to
144 distinguish: i) dry grasslands and hay meadows (shrub presence not detectable), ii) mixed
145 typologies (dry grasslands/hay meadows with shrub cover < 50%) and iii) shrubland (shrub
146 cover > 50%). Using also the historical knowledge concerning the landscape management
147 provided by the local administrator, we defined the sampling layers as follow: 1) Habitat
148 (corresponding to the land use) distinguishing between dry grasslands and hay meadows and 2)
149 Succession, distinguishing between i) active (hereafter Managed) ii) short-time abandoned
150 (hereafter Transition) and iii) long-time abandoned areas (hereafter Abandoned). The stage of
151 succession is used as a proxy for the time from abandonment of the typical land management
152 practices, considering that an increase of the cover of shrubs corresponds to the increase of time
153 following abandonment. Hence, we performed a random selection of 10 square plots of 2x2
154 meters for each stratum in a full factorial design, leading to 60 sampled plots (10 x 2 types of
155 habitat x 3 stages of succession). In each plot, we performed a floristic sampling, recording the
156 presence/absence of vascular plants. Voucher specimens were collected for identification in the
157 laboratory, and further samples were collected for the genetic analyses.
158
Selection of molecular markers, DNA isolation, sequence alignment and tree reconstruction

160 Phylogenetic diversity of the spermatophyte communities was inferred from the analysis of three
161 markers of the nuclear ribosomal DNA, the ITS1-5.8S and ITS2 regions. These markers, widely
162 used for phylogenetic studies both in plants and fungi, have shown a great discriminatory power
163 at low taxonomic levels (Feliner and Rosselló 2007; Hollingsworth *et al.* 2011), supporting this
164 region as a core barcode for spermatophytes (Li *et al.* 2011) and hence potentially usable as a
165 proxy for evolutionary relationships. We assembled a sequence dataset by retrieving accessions
166 of the sampled species from GenBank (http://www.ncbi.nlm.nih.gov/) to construct a tree
167 resolved at the species level. Molecular analyses were performed for 13 species for which no
168 accessions were available in the GenBank. Isolation of genomic DNA followed a modified
169 2×CTAB protocol successfully adopted in previous studies using molecular tools (see Coppi *et*
170 *al.* 2008 and Appendix S1 for further details).
171 The final dataset of the sampled species consisted of 147 accessions (Table S2). Taxa from
172 Gnetales, Ginkgoales, Cycadales, Pinales and Cupressales were added in order to obtain a correct
173 alignment for Gymnosperms, whereas four taxa from Polypodiales and Salviniales were added as
174 outgroups (Table S1). Multiple alignment of the ITS-5.8S dataset was performed with MAFFT
175 (v. 7.0, Katoh and Standley 2013) adopting the parameterization typically used for nucleotide
176 sequences (200PAM/k=2, gap penalty=1.53; offset=0.0), considering that we aligned very
177 distant species. We followed a step by step multi-alignment procedure: (1) taxa were grouped at
178 the order level and aligned using the Q-INS-i strategy, checking each multi-alignment by visual
179 inspection with BioEdit; (2) the multi-alignments were merged at higher ranks using the *merge*
180 option in MAFFT, obtaining separate multi-alignments for Eudicots, Monocots, Gymnosperms
181 and for the outgroup; (3) these four multi-alignments were finally merged again. The alignment
182 was used to build a phylogenetic tree with a maximum likelihood (ML) approach by means of
183 RAxML (Stamatakis, 2006) via the CIPRES supercomputer cluster (http://www.phylo.org/),
184 using 1000 maximum searches. The topology of phylogenetic inference was constrained at the
185 family level using as backbone the tree *slik2015* (Slik *et al.* 2018) available in Phylomatic vers. 3
186 (http://phylodiversity.net/phylomatic/). This topology is mainly based on the APG III phylogeny,
187 further resolved up to genus level using the species-level phylogeny in Zanne *et al.* (2014),
188 placing at the base of their respective families genera not present in Zanne *et al.* (2014), see Slik
189 *et al.* (2018). The resulting topology is highly consistent with the hypothesis in Magallón *et al.*
190 (2015), which was subsequently used to date the phylogeny, except for the clade involving the
191 *Malpighiales* and the one involving *Boraginales*, *Solanales*, and *Gentianales*. For these clades
192 we followed the tree *slik2015*. The statistical support to the nodes was estimated using the
193 bootstrap method (1000 iterations). Finally, to obtain an ultrametric tree, we calibrated our
194 phylogeny dating the node ages according to Magallón *et al.* (2015), adopting a Molecular
195 Dating approach throughout Penalised Likelihood estimation via the *chronos* function of ape
196 v5.1 R package (Paradis *et al.* 2004).
197
198 **Evaluation of trends in species richness and phylogenetic relatedness (α-diversity)**

199 We evaluated the changes in taxonomic species richness (SR) and in three indices allowing the
200 assessment of different features of phylogenetic α-diversity. We used the Phylogenetic Diversity
201 (PD) as a measure of the amount of phylogenetic richness in the communities (how much) and
202 the Net Relatedness Index (NRI) and the Nearest Taxon Index (NTI) to provide information
203 regarding the phylogenetic divergence within the communities (how different they are) (see
204 Tucker *et al.* 2017).
205 Faith's PD (Faith 1992) represents the simplest measure of the cumulative evolutionary age in a
206 community, but it is highly correlated to species richness. Thus, we adopted its Standardized
207 Effect Size index that is generally considered unaffected by species richness (Pavoine *et al.*
208 2013; Swenson 2014; but see Sandel 2018), and indicates whether the observed PD is different
209 from what would be expected by chance. To allow an interpretation of results comparable with
210 NRI and NTI, we multiplied PD.ses by -1. We defined NTI and NRI as Standardized Effect Size
211 indices of Mean Nearest Taxon Distance (MNTD) for taxa in a community and Mean Pairwise
212 Distance separating taxa in a community (MPD). MNTD is calculated as the mean of the
213 smallest non-diagonal value in the pairwise distance matrix for each species and is a measure of
214 the branch-tip phylogenetic clustering of the species in the community (Webb *et al.* 2002). It
215 describes the phylogenetic relatedness among species, focusing on the distal part of the tree, thus
216 involving lower taxonomical levels. MPD estimates the average phylogenetic relatedness
217 between all possible pairs of taxa in an assemblage, calculated as the mean of the non-diagonal
218 elements in the pairwise distance matrix (Webb 2000). It is a measure of the relationship at the
219 higher-level groups in the phylogenetic tree. NTI and NRI indicate whether the values of MPD
220 and MNTD differ from what would be expected by chance. Positive values of NRI and NTI
221 indicate that observed phylogenetic distances are lower than expected and that phylogenetic
222 clustering of species occurs. Conversely, negative values of such indices indicate phylogenetic
223 over-dispersion or evenness. In general terms, NTI and NRI values higher/lower than 1.96/-1.96
224 are usually considered indicators of significant patterns. All standardized indices (PD.ses, NRI
225 and NTI). All standardized effect sized indices (PD.ses, NRI and NTI) were calculated using a
226 comparison with *fixed-fixed* null models, which maintain both species richness and species
227 abundance across sites and tend to exhibit low type I and II error rates (see Miller et al., 2017).
228 The null model matrices were randomized using the "independent-swap" algorithm by Gotelli
229 (2000), which is well suited for presence/absence community matrices.
230 We studied the variation SR and PD.ses, NTI and NRI in a two-way ANOVA design considering
231 Habitat and Succession as explanatory variables, also taking into account their interaction effect.
232 We further explored the differences in the levels of the significant terms performing a Post-Hoc
233 Tukey Test.
234 To assess the role of *Junipers communis* in the phylogenetic α-diversity patterns found, we
235 repeated the analyses on PD.ses, NTI and NRI excluding this species, and keeping the same
236 design described above.
237
238 **Trends of compositional shifts (β-diversity)**

239   To assess the variations in species composition of plots, we ran a comparison among a detrended
240   correspondence analysis (DCA) and a canonical correspondences analysis (CCA) on the plot
241   species composition. Thus, we checked the efficiency of constrained axis to catch the variation
242   explained by unconstrained axis, as provided in the software Canoco 5 (ter Braak and Šmilauer
243   2012) and following Šmilauer and Lepš (2014). In DCA the axes were detrended by segment,
244   adopting default options in Canoco 5 In the CCA, Habitat and Succession were used as
245   explanatory variables and the significance of the constrained axes was tested with 4999
246   unrestricted permutations.
247   We used the methodological framework developed by Podani and Schmera (2011) to evaluate
248   the trends in β-diversity components during the succession. This methodology allows the
249   partition of pairwise gamma diversity into three complementary indices, measuring Similarity,
250   relative Richness Difference and relative Species Replacement (respectively S, D and R), and
251   accordingly is referred to as SDRSimplex approach (see also Appendix S2 for further
252   description). Pairwise-comparisons regarded plots of the same habitat, spanning along the
253   succession. The SDRSimplex results were projected in a ternary plot. Finally, we used a
254   Nonparametric Kruskal–Wallis test to check the significance of the differences among habitats.
255   To assess the role of particular species in the species turnover, we carried out an Indicator
256   Species Analysis (ISA, Dufrêne and Legendre 1997). The ISA allows computing an indicator
257   value d (ranging between 0 and 100) of each species as the product of the relative frequency and
258   relative average abundance of species in clusters. The analysis also produces a significance
259   value, representing the probability of obtaining a d value as high as that observed over 1000
260   iterations. We conducted the analyses considering each stage of succession of the two habitats as
261   a separate cluster.
262   We used a three dimension Non-Metric Multidimensional Scaling (NMDS) ordination based on
263   the UniFrac index distance matrix, to assess how different were the communities from the
264   phylogenetic point of view. UniFrac is a phylogenetic diversity-based dissimilarity index that
265   measures the proportion of evolutionary history unique to each community and is calculated as
266   the total branch length unique to each community relative to the total branch length linking all
267   species in both communities (Lozupone and Knight 2005).
268   Furthermore, we studied the evolutionary dissimilarity between communities along the
269   succession (phylogenetic β-diversity), adopting the approach described in Leprieur *et al.* (2012)
270   as an improvement of PhyloSor index. This index expresses to what extent the compared
271   communities are composed by related species rather than by species that share no branch in the
272   phylogeny and can be separated in two components accounting for 'true' phylogenetic turnover
273   (PhyloSor$_{Turn}$) and phylogenetic diversity gradients (PhyloSor$_{PD}$). In addition, we also analyzed
274   the standardized effect size of such indices (i.e. SES.PhyloSor, SES.PhyloSor$_{Turn}$ and
275   SES.PhyloSor$_{PD}$), which describe whether two communities are phylogenetically more or less
276   dissimilar than what is expected given their taxa dissimilarity. These indices are obtained via
277   comparison with a null model in which species are randomized across the tips from the tree
278   while holding constant species richness and compositional beta diversity in 999 simulations.

279 Again, we used a Nonparametric Kruskal–Wallis test to check the significance of the differences
280 among the two habitat types.
281 Furthermore, to assess the role of *Junipers communis* in the phylogenetic β-diversity patterns
282 found, we repeated the analyses excluding this species, and keeping the same design described
283 above.
284 All ordination analyses (DCA, CCA and NMDS) and relative graphs were made using the
285 software Canoco 5 vers. 5.12 (ter Braak and Šmilauer 2012). All other analyses were made using
286 R software (version 3.5, The R Foundation for Statistical Computing, Vienna, Austria, www.R-
287 project.org), and relative graphs were produced with ggplot2 vers. 2.2.1 (Wickham 2009). The
288 ISA was conducted using the package labdsv (R package version 1.8-0, https://CRAN.R-
289 project.org/package=labdsv). Phylogenetic β-diversity indices were calculated exploiting the R
290 functions developed by Leprieur *et al.* (2012). Phylogenetic α-diversity metrics were obtained
291 with the package metricTester vers. 1.3.6 (Miller et al., 2017).
292
293 **Results**
294
295 The sampling resulted in 147 species (Table S2), with 69 shared species among the two habitats
296 and 39 species exclusive to each habitat (tot. 108 species in both habitats). Species richness
297 varied from 5 to 29 species per plot, in Abandoned dry grasslands and Managed dry grasslands,
298 respectively. The resulting phylogenetic tree is shown in Figure S1 (see Appendix S3 for the tree
299 in Newick format).
300 Species richness was comparable between the two habitats and decreased in both during the
301 succession (Table 1, Fig. 1A). Conversely, PD.ses differed significantly among the habitats, with
302 lower values in dry grasslands, and during the succession, with a decrease in the latter stage
303 (Table 1, Fig. 1B). Regarding NRI, the two habitats showed different trends during the
304 succession (significant interaction Succession x Habitat, Table 1). Indeed, while they displayed
305 comparable values at earlier succession stages with values indicating random processes of
306 species selection, dry grasslands shifted in the latter stage to a significant overdispersion of plant
307 composition, with the mean NRI value below the critical threshold of -1.96 and significantly
308 different from the one of hay meadows (Fig. 1C). On the other hand, the two habitats displayed a
309 comparable NTI trend during the succession, with values in hay meadows generally higher
310 (Succession and Habitat both significant, but no significant interaction, Table 1).
311 Notwithstanding a significant drop in the latter stages of succession, in all three stages the mean
312 NTI values remained between +/- 1.96, again indicating random processes of species selection
313 (Fig. 1D).
314 The analyses concerning phylogenetic α-diversity conduced excluding *J. communis* showed
315 quite a different scenario. PD.ses varied significantly between the two habitats and showed
316 different trends during the succession (Interaction term Succession x Habitat significant, see
317 Table 2), while both NTI and NRI varied significantly only along the succession with
318 comparable trends between the two habitats (Table 2). Indeed, we recorded a dramatic rise in

319   PD.ses in abandoned dry grasslands compared to what happened considering *J. communis*. As a
320   result, PD.ses in dry grassland is more or less stable along the succession, while it vary
321   significantly in hay meadows (Fig 2A). On the contrary, all patterns toward a phylogenetic
322   overdispersion in dry grasslands clearly vanished, with both NTI and NRI higher than those *J.*
323   *communis* and even closer to 0 than those of hay meadows, thus with a net predominance of
324   random processes of species selection (Fig 2 C, D).
325   The species composition of two habitats resulted clearly separated along the succession. CCA
326   constrained axes showed a very good efficiency, catching 85.5% and 100% of variation
327   explained by CA unconstrained axis. Total variation is 4.87, DCA first two axes explained
328   10.90% and 6.90% of it, while CCA ones 9.26% and 6.93% (P<0.001). Plots resulted clearly
329   differentiated in terms of species composition with a clear set of taxa specific to each habitat and
330   each stage, with changes during the succession lying on the horizontal axis and differences
331   among the two habitats on the vertical one (see Fig 3A and B). According to the ordinations, the
332   two habitats show common trends of species replacement, with new species coming in the
333   transition stage and a further differentiation in the last stage.
334   The pairwise comparisons across the two habitats highlighted significant differences in the trends
335   in species turnover among the succession. Indeed, they shared consistently low values of
336   similarity (S) but were characterized by significantly different values of species replacement (R)
337   and richness difference (D). In particular, dry grasslands displayed a higher D and a lower R than
338   hay meadows (Fig. 4).
339   The ISA confirmed the presence of different trends in the numbers of species characterizing the
340   succession stages and leading the succession. In the managed stage, the two habitats shared the
341   same number of indicator species, but hay meadows presented a higher number of indicators
342   species than dry grasslands in the following stages (17, 5, and 9 species in hay meadows vs. 17, 3
343   and 1 in dry grasslands in the Managed, Transitional and Abandoned stages, respectively, see
344   table S3).
345   The NMDS ordination on phylogenetic distance among plot allowed a good representation of the
346   UniFrac distances (Stress criterion = 0.141). As for DCA and CCA, in the NMDS first axis
347   reported the changes during the succession, while the habitat are separated along the second axis.
348   Here, particularly the transitional stage of hay meadows show a high similarity with managed
349   and transition stages of dry grasslands, while again the two habitats showed a relevant
350   differentiation in the latter stage (Fig. 5).
351   Phylogenetic β-diversity was comparable among the two habitats and in both communities was
352   mainly composed by phylogenetic turnover (Fig 6A). Only PhyloSorPD was significantly
353   different between the two habitats, being higher in dry grasslands than in hay meadows. None of
354   the phylogenetic β-diversity standardized metrics differed significantly from what is expected
355   given the taxa dissimilarity (all values between +/- 1.96, see fig 6C). Nevertheless, both
356   SES.PhyloSor and the component SES.PhyloSorPD resulted significantly lower in dry grasslands
357   than in hay meadows. The analyses conducted excluding *J. communis* from dry grasslands
358   resulted in higher values of PhyloSor$_{PD}$ and lower ones of PhyloSorPD, both differing

359 significantly between the two habitats (fig 6B). SES.PhyloSor$_{Turn}$ also showed significantly lower
360 values in dry grasslands (fig 6D).
361
362 **Discussion**
363
364 The two habitats showed different features characterizing the changes in species composition
365 during the succession, even if they displayed a comparable tendency in species richness loss. The
366 trend in species loss has a long history of detection in the succession following the abandonment
367 of secondary grasslands (Corazza *et al.* 2016; Csergő *et al.* 2013; Dengler *et al.* 2014; Janišová *et*
368 *al.* 2011; Rocchini *et al.* 2006). Concerning Apennine hay meadows, Ubaldi (2003) reported that
369 when agricultural activities are abandoned, soil water capacity and structure decreases.
370 Consequently, also hay production decreases and these areas are used as pastures. Meanwhile,
371 trampling and grazing further reduce water capacity and fertility of soils, so that they become dry
372 grasslands, more or less xerophilous depending on the site. However, this general trend is not
373 consistent with our findings, which highlighted a clear divergence of the two habitats during the
374 succession.
375 The values of PD.ses showed that whether in managed and transition stages the PD is
376 substantially consistent to what should be expected given the taxa richness, in the abandoned
377 ones PD values were higher than expected. This indicate that whether SR decreased, PD do not
378 decreased consistently, because the loss in species seems to be counterweighted by the presence
379 of species with long branches in the phylogeny (and this is particularly true for dry grasslands).
380 Regarding phylogenetic structure, we detected for the first succession stages a predominant role
381 of random processes of species loss in both habitats. These trends may be consistent with a
382 framework in which a random phylogenetic structure of the community is the result of
383 competitive exclusion of species in the case of convergent traits (see Davies 2006). Nevertheless,
384 we detected a net difference of the community structure concerning the deep nodes of
385 phylogeny, linked to the overdispersion in the dry grasslands. This is the result of the reduction
386 of the number of species and the appearance (as a dominant participant) of the stress-tolerant
387 species *J. communis* (Pierce *et al.* 2017). This is in strong agreement with our hypothesis that
388 these environments are dominated by a strong component of ecological stress. Accordingly,
389 species assembling processes may have selected for traits allowing to survive in xeric
390 environments (i.e. traits linked to conservative economics in the leaf economics spectrum, such
391 as small and thick leaves, low growth rate, small specific leaf area and high leaf dry matter
392 content), that in this case were shared between species distant in the phylogeny.
393 The analysis of β-diversity trends confirmed these differences. In both habitats, we evidenced a
394 strong loss in species, but with a high component of richness difference in dry grasslands,
395 indicating a smaller replacement by new species. These findings match with those of several
396 authors, who showed how short species are outcompeted by dominant tall grasses in the first
397 succession stages after the abandonment (Corazza *et al.* 2016), leading, in agreement with Grime
398 (2001), to the exclusion of subordinate and accidental species. Furthermore, in the latter

399   succession stages many more species are lost from the community with the dominance of *J.*
400   *communis*. Also in hay meadows, the loss in species following the abandonment was
401   characterized by a suppression of species, but with a major turnover of species. Accordingly,
402   Csergő *et al.* (2013) demonstrated that the loss in species following the meadows abandonment
403   may be driven by the suppression of dominant grasses by tall forbs, in meadows co-dominated
404   by competitive stress-tolerant ruderals, whereas in meadows dominated by a single stress-
405   tolerant competitor, diversity loss resulted from increased abundance and biomass of the
406   dominant grass.
407   These trends were confirmed by ISA results, which are consistent with the scenario outlined in
408   the DCA and CCA scatterplots. The higher rate of richness difference, and the lower species
409   replacement of dry grasslands, in particular emerged from the low number of species
410   characterizing transition and abandoned stages. Indeed, in the managed stage, indicator species
411   resulted numerically comparable among the two habitats. In managed hay meadows, the
412   indicator species belong mainly to the families *Poaceae* and *Fabaceae* (*Lolium perenne, Phleum*
413   *bertolonii, Bromus hordeaceus, Cynosurus cristatus, Trifolium pratense, Vicia sativa* and
414   *Lathyrus pratensis*), strictly linked to the pastoral activities, or consisted of other mesotrophic
415   plants favored by grazing (i.e. *Ranunculus bulbosus*). On the other hand, the indicator species of
416   managed dry grasslands were more typical of shallow and rocky soils, such as *Thymus*
417   *longicaulis, Acinos alpinus, Trifolium scabrum, Bupleurum baldense* and *Cerastium*
418   *brachypetalum*. In the Transition, hay meadows were characterized by a higher number of
419   indicator species, with species typical of open habitats (*Centaurea nigrescens, Achillea collina,*
420   *Poa trivialis, Dactylis glomerata, Cirsium tenoreanum*). These features are consistent with the
421   scenario described above following Csergő *et al.* (2013). Transition dry grasslands were
422   characterized by the dominance of *Brachypodium rupestre*, (together with plants considered
423   precursory of more closed and woody habitats, see e.g. Assini *et al.* 2014). The dominance of
424   *Brachypodium* spp. in successions post-abandonment is a general trend widely demonstrated for
425   Apennine grasslands (Corazza *et al.* 2016). Finally, *J. communis* resulted the sole indicator
426   species of the abandoned dry grasslands, while abandoned hay meadows were characterized by a
427   high number of indicator species, spanning from various woody species of several families (i.e.
428   *Rosaceae, Salicaceae, Aceraceae, Oleaceae*) to some herbaceous plants (for example
429   *Ranunculus lanuginosus* and *Valeriana officinalis*).
430   The analysis of phylogenetic distances among plots highlighted again that while the two habitats
431   were well differentiated in their composition (also in term of species lineages) in the managed
432   stage, during the succession the replacement of lineages in the communities brought to a certain
433   degree of phylogenetic similarity between managed hay meadows and both managed and
434   transition dry grassland. Nevertheless, as the succession proceeded further, the species
435   composition of abandoned grasslands led to a net differentiation of the two habitats. Indeed, the
436   two habitats hosted some species in common (or at least some species sharing common lineages)
437   in the transitional stage (as also highlighted by the CCA). Subsequently they differentiated again
438   in the last stage, with a higher replacement in hay meadows and dry grassland more or less

439   dominated by a specie (*J. communis*) not present in hay meadows and phylogenetically distant
440   from all other species.
441   As to phylogenetic β-diversity, both habitats showed a greater contribution of 'true' phylogenetic
442   turnover (reflected in a minor importance of phylogenetic gradient). These results indicated that
443   the difference among plots was due to the replacement of species coming from different lineages,
444   rather than from a simple difference in PD. Nevertheless, in both the cases of the simple and the
445   standardized indices, we recorded differences concerning the amount of beta diversity deriving
446   simply from a difference in PD. This component is numerically higher in dry grasslands
447   (referring to simple Phylosor.PD) but is lower than what could be expected given the taxa
448   dissimilarities when looking at the SES.Philosor$_{PD}$. We can hypothesize that this dependence
449   may be related to the presence in dry grasslands of lower species replacement, with the entrance
450   of a species phylogenetically very distant from the others (*J. communis*), which balances out the
451   importance of simple PD component.
452   The pivotal role of *J. communis* in dry grasslands emerged on re-running all analyses concerning
453   phylogenetic α- and β-diversity excluding this species. This species resulted responsible for a
454   high amount of PD.ses in dry grasslands, and also the main one responsible for the presence of
455   patterns of overdispersion. Also looking at -diversity, once the balancing effect exerted by *J.*
456   *communis* had been removed, the PhyloSor$_{PD}$ resulted even higher. In addition, the SES.PhiloSor
457   component rose significantly, showing that the amount of SES.PhiloSor$_{PD}$ was substantially
458   consistent to what could be expected given the taxa dissimilarities and is higher than the one
459   observed in hay meadows. In addition, differences among the two habitats in both PhyloSor$_{Turn}$
460   and SES.PhyloSor$_{Turn}$ became significant when excluding *J. communis*, indicating firstly a lower
461   replacement of lineages in dry grasslands, balanced by the bigger contribution of PhyloSor$_{PD}$ and
462   secondly that replacement in dry grasslands was driven by species sharing a closer lineage than
463   those in hay meadows. These results highlighted the important role of *J. communis* and
464   pinpointed the importance of including this species in the analyses, also considering that this is a
465   key species in late succession stages of dry grasslands, being the dominant one, but also that its
466   presence may hide phylogenetic differences between habitats driven by other clades. It is also
467   noteworthy that, whereas it is common to remove highly phylogenetic distinct species from
468   phylogenetic analysis, this practice may lead to a partial understanding of the processes at work,
469   and that an in-depth interpretation of phylogenetic patterns should be made both using or not this
470   distantly related species.
471   In this study, we used presence/absence data, and it should be acknowledged that abundance data
472   may have led to significantly divergent results. Even if in our case, one of the main species
473   responsible for the recorded patterns was a very abundant and dominant one. Nevertheless,
474   further studies including abundance data are necessary to better depict the processes at work.
475
476   **Conclusions**
477

478   In conclusion, our data elucidate the differences in the secondary succession of dry grasslands
479   and hay meadows in the Tuscan Apennines. In both cases, we recorded a drop in taxonomic α-
480   diversity during the succession, but the analyses of taxonomic β -diversity highlighted quite
481   different compositional changes, with dry grasslands mainly dominated by richness difference
482   and hay meadows characterized by higher species replacement. As regards the phylogenetic
483   patterns, we were able to verify that they followed a comparable trend in the earlier succession
484   stages of the two habitats, but the entrance of a single species characterized by a deep separation
485   in the phylogeny of the communities (i.e. *J. communis*) raises substantial differences. We
486   propose an important role of the ecological factors in these trends, with the selection of *J.*
487   *communis* fostered by a dominance of abiotic filters and resulting in the outcompeting of
488   subordinate and accidental species in the latter stage of the habitat succession in dry grasslands,
489   after an initial dominance of competitive exclusion of the species. Nevertheless, as noted in the
490   introduction, the assessment of links among phylogenetic patterns and ecological processes
491   needs more in-depth study. Our proposed scenario could be appropriate in the case of traits not
492   conserved in the phylogeny (and this could be in accordance with the idea that trait
493   conservativism should not be taken for granted, see Gerhold *et al.* 2015), but a certain evaluation
494   of plant traits is necessary to further explore such trends, especially considering the concerns
495   related to the use of phylogeny as proxies for community assembly mechanisms.
496

Acknowledgements

498
499   We wish to thank the Raggruppamento Carabinieri Biodiversità of the Reparto Carabinieri
500   Biodiversità Pieve Santo Stefano, for its support. We also wish to thank the Editor Cajo ter
501   Braak, Lars Götzenberger and one anonymous reviewer whose suggestions greatly helped
502   improve the manuscript.
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Analysis of variance table for the effect of Habitat and Succession on indexes of taxonomic and phylogenetic α-diversity.

Species Richness (SR), Standardized Effect Size of Faith's Phylogenetic Diversity (PD.ses), Net Relatedness Index (NRI) and Nearest Taxon Index (NTI). Significance codes: P value < 0.001 "***"; P value < 0.01 "**"; P value < 0.05 "*")

1   **Table 1.**

Response	Factors	Df	ResDf	Sum Sq	F Value	P value	
SR	Habitat	1	54	22.82	2.067	0.156	
	Succession	2	54	1930.23	87.458	<0.001	***
	Habitat: Succession	2	54	54.03	2.448	0.096	
PD.ses	Habitat	1	54	9.49	8.34	0.006	**
	Succession	2	54	55.60	24.43	<0.001	***
	Habitat: Succession	2	54	1.87	0.83	0.440	
NRI	Habitat	1	54	21.33	17.77	<0.001	***
	Succession	2	54	50.27	21.33	<0.001	***
	Habitat: Succession	2	54	17.08	7.11	0.002	**
NTI	Habitat	1	54	3.16	2.92	0.093	
	Succession	2	54	48.82	21.19	<0.001	***
	Habitat: Succession	2	54	1.29	0.64	0.554	

Analysis of variance table for the effect of Habitat and Succession on indexes of phylogenetic α-diversity evaluated excluding from the analyses the species *Juniperus communis.*

Standardized Effect Size of Faith's Phylogenetic Diversity (PD.ses), Net Relatedness Index (NRI) and Nearest Taxon Index (NTI). Significance codes: P value < 0.001 "***"; P value < 0.01 "**"; P value < 0.05 "*".

1   **Table 2.**

Response	Factors	Df	ResDf	Sum Sq	F Value	P value	
	Habitat	1	54	0.50	0.58	0.451	
PD.ses	Succession	2	54	28.29	16.30	<0.001	***
	Habitat: Succession	2	54	11.03	6.36	0.003	**
	Habitat	1	54	0.39	2.05	0.158	
NRI	Succession	2	54	7.33	19.03	<0.001	***
	Habitat: Succession	2	54	1.00	2.61	0.083	
	Habitat	1	54	0.01	0.01	0.920	
NTI	Succession	2	54	25.69	17.09	<0.001	***
	Habitat: Succession	2	54	4.18	2.78	0.071	

Figure 1

Interaction plots for the variation in taxonomic and phylogenetic α-diversity of the 60 sampled plots according to Habitat and Succession.

(A) Species Richness (SR). (B) Standardized Effect Size of Faith's Phylogenetic Diversity (PD.ses). (C) Net Relatedness Index (NRI). (D) Nearest Taxon Index (NTI) Different letters indicate significant differences (p<0.05) after a Post Hoc Tukey's test conducted in A, B and D) between the levels of the factor Succession, and in C) between the levels of the interaction Succession*Habitat, according to the ANOVA results.
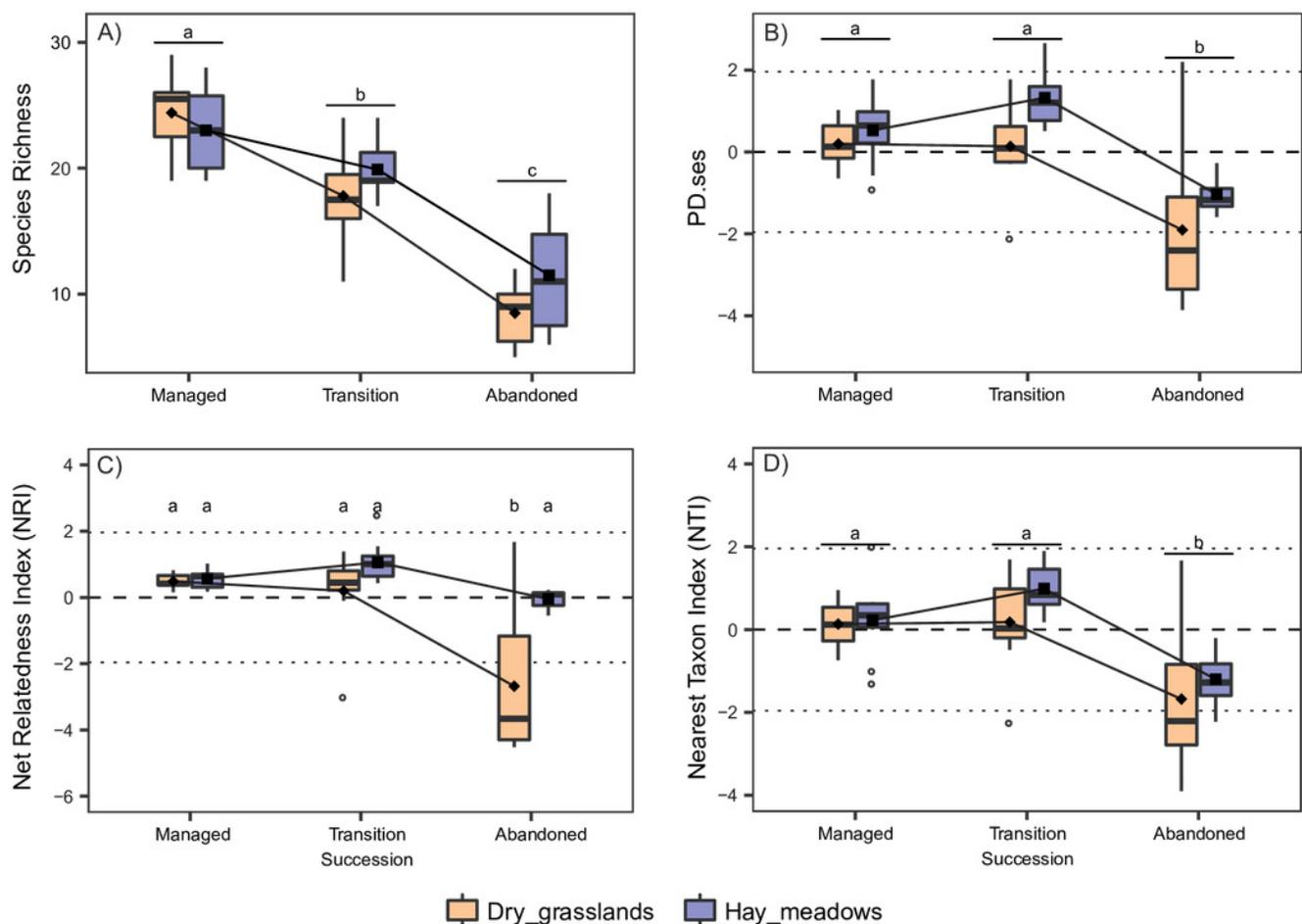
Figure 2

Interaction plots for the variation in phylogenetic α-diversity of the 60 sampled plots according to Habitat and Succession evaluated excluding from the analyses the species *Juniperus communis*.

(A) Standardized Effect Size of Faith's Phylogenetic Diversity (PD.ses). (B) Net Relatedness Index (NRI). (C) Nearest Taxon Index (NTI). Different letters indicate significant differences (p<0.05) after a Post Hoc Tukey's test conducted in A) between the levels of the interaction Succession*Habitat, and in B and C) between the levels of the factor Succession, according to the ANOVA results.
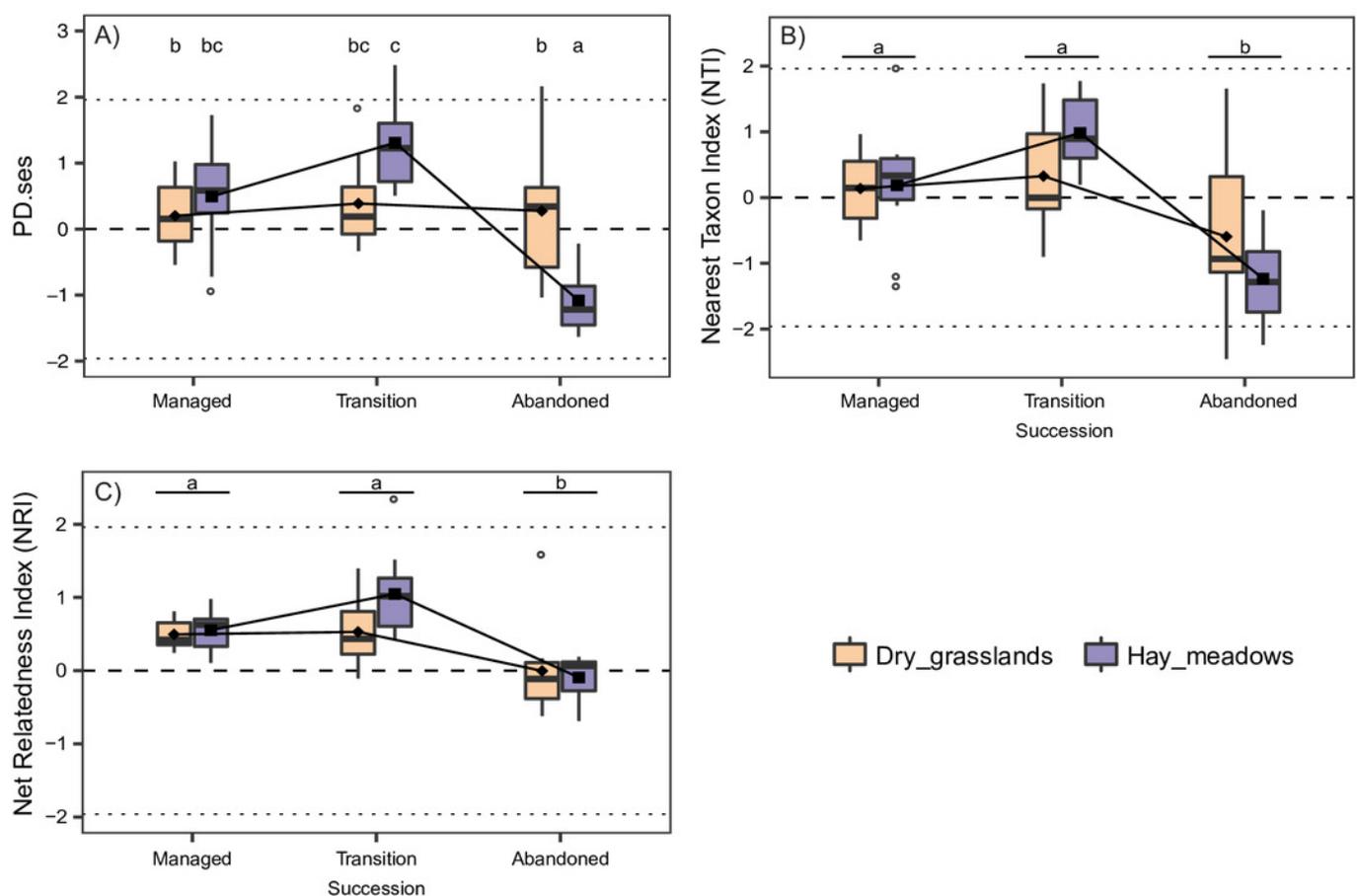
Figure 3

Ordination diagrams considering species composition of plots.

A) Plot distribution according to DCA considering their species composition. Empty symbols represent hay meadows and full symbols represent dry grasslands. Rhombus represent managed plots, circles transition plots and squares abandoned ones. B) Plant species distribution obtained with CCA, only 50 best fitting species are shown, see also table S3 for indicator species. Blue triangles represent the species. In both graphs, red triangles represent plot centroids according to Habitat type and Stage of Succession. H-MN= managed hay meadows, H-TR= transition hay meadows, H-AB= abandoned hay meadows, D-MN= managed dry grasslands D-TR= transition dry grasslands D-AB= abandoned dry grasslands.
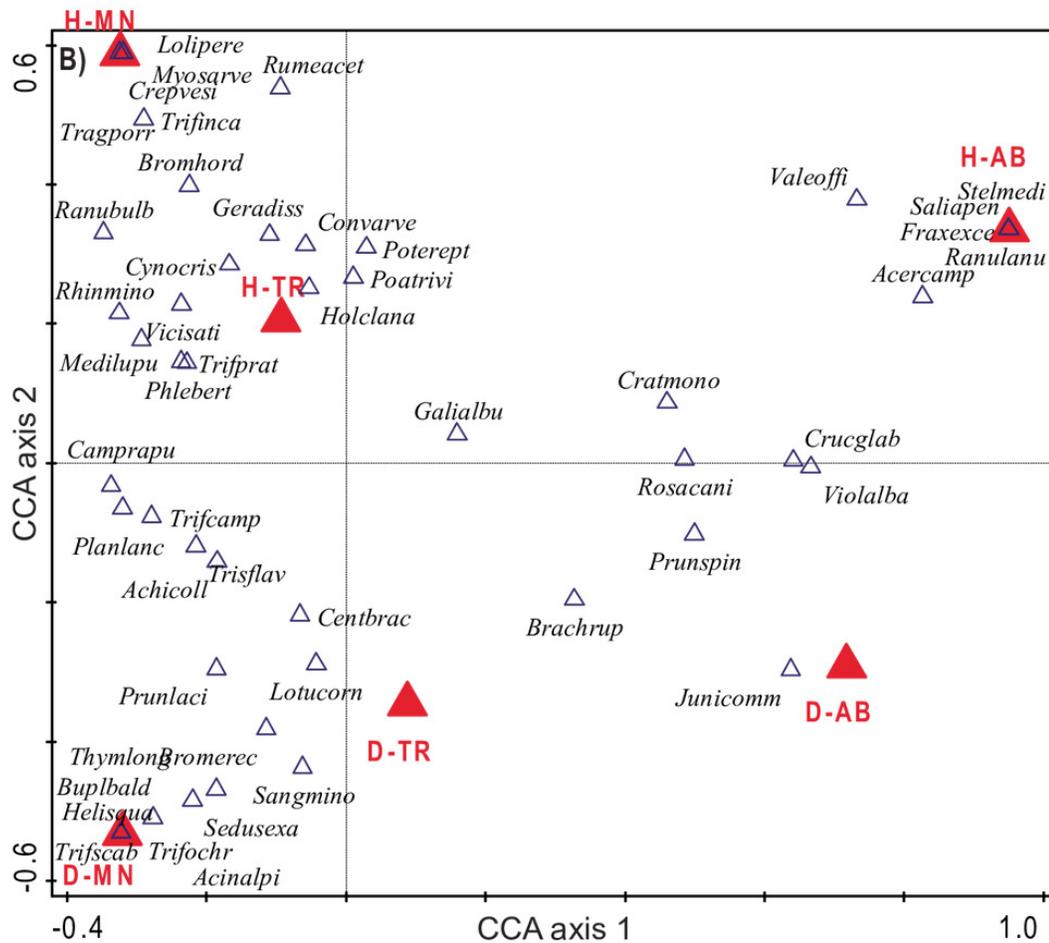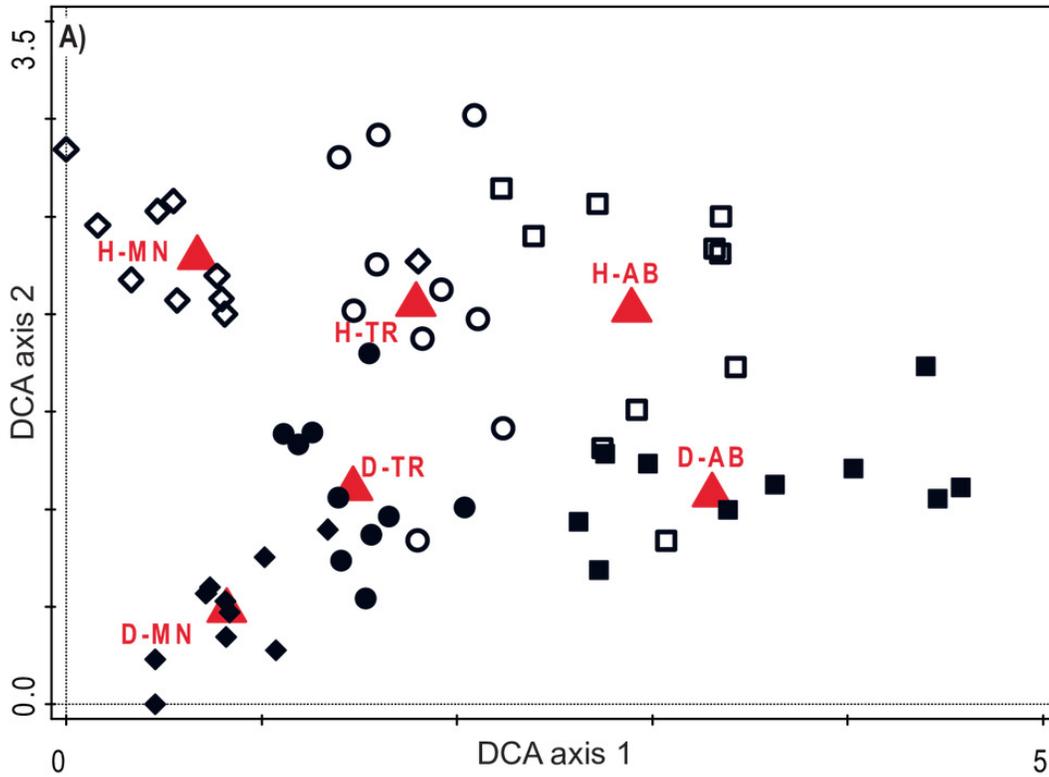
Figure 4

SDR simplex ternary plots showing the variation in taxonomic β-diversity along the seconadary succession of the two habitats.

(A) Dry grasslands. (B) Hay meadows. The abbreviations S, D and R refer to similarity, richness difference and species replacement, respectively. Mean values of S, D, and R are reported. Values marked with * are significantly different at P<0.05 according to a Kruskal-Wallis test performed between the two habitats.
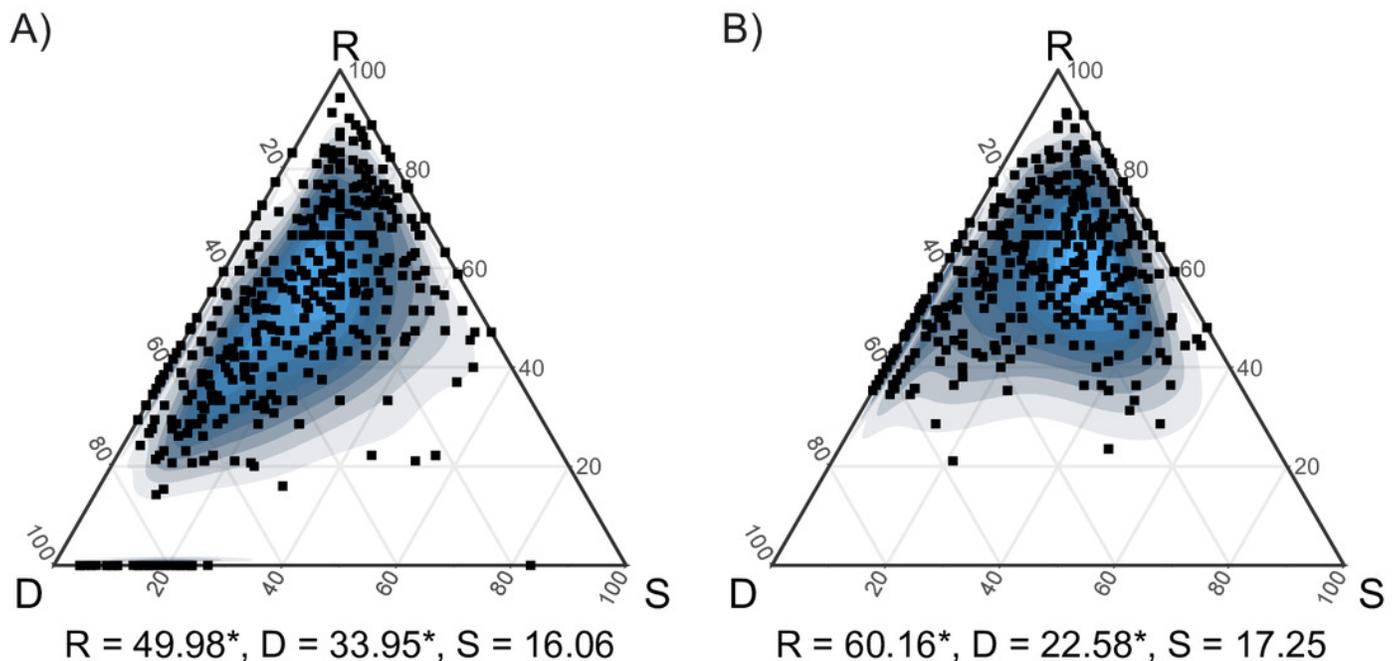
Figure 5

Ordination diagram based on phylogenetic distance of plots.

Plot distribution according to NMDS ordination based on the UniFrac index distance matrix. Empty symbols represent hay meadows and full symbols represent dry grasslands. Rhombus represent managed plots, circles transition plots and squares abandoned ones. Convex hull envelopes enclose plots according to Habitat type and Stage of Succession.
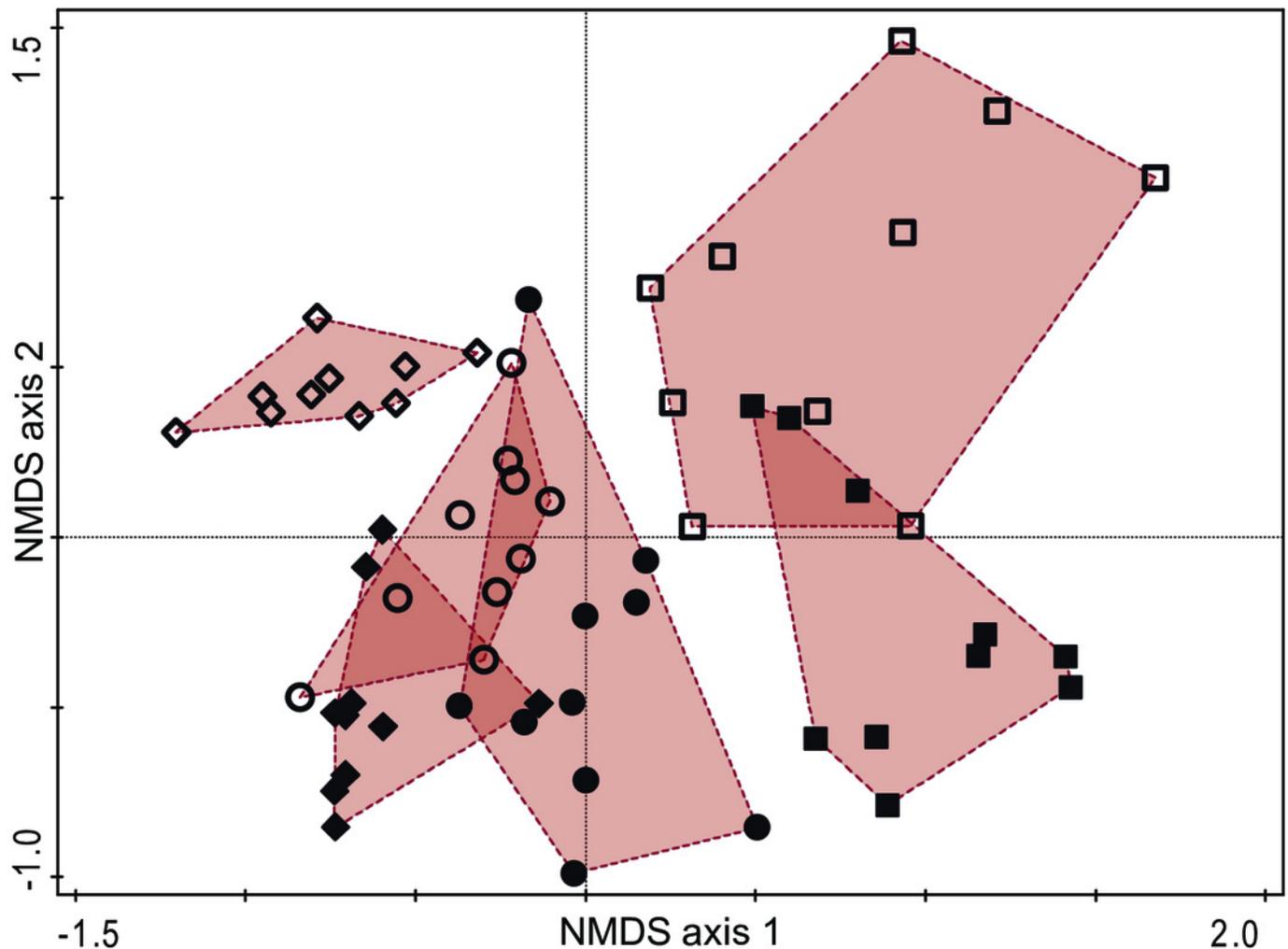
Figure 6

Boxplot graph for the variation in phylogenetic β-diversity according to Habitat and Succession for dry grasslands and hay meadows during the succession, evaluated with and without the species *Juniperus communis*.

A and B) PhyloSor index, and its two separate components accounting for 'true' phylogenetic turnover (PhyloSorTurn) and phylogenetic diversity gradients (PhyloSorPD) evaluated with and without the species *J. communis*, respectively; C and D) their relative Standardized Effect Size (SES.PhyloSor, SES.PhyloSorTurn, SES.PhyloSorPD) again evaluated with and without the species *J. communis*, respectively. Couples of boxplot marked with * show data significantly different at P < 0.05 according to a Kruskal-Wallis test performed between the two habitats.