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1 **Soil chemical properties in abandoned Mediterranean cropland after**  
2 **succession and oak reforestation**

3

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16

17

18 **Abstract**

19 Large extents of cropland have been abandoned in recent decades and more may be  
20 abandoned in the near future. **These** may undergo secondary succession or reforestation.  
21 We experimentally tested the response of soil chemical properties to secondary  
22 succession (old field) and to *Quercus ilex* plantation (reforested cropland) in  
23 Mediterranean cropland that was abandoned 13 years ago. We also evaluated the  
24 relevance of previous reforestation management (four combinations of presence and  
25 absence of irrigation and shading) in addition to current environmental conditions  
26 (herbaceous community and cover of oak canopy) on soil chemistry in the reforested  
27 cropland. Carbon and  $\text{NH}_4^+$ -N concentrations and availability of mineral N were higher  
28 in the reforested cropland than in the old field. However, soil pH, total N, P, K and  $\text{NO}_3^-$   
29 -N concentrations, mineralization rates, and available  $\text{PO}_4^{3-}$ -P were similar in the  
30 reforested cropland as well as in the old field. Previous management practices,  
31 particularly irrigation, and current environmental conditions, mostly biomass and  
32 composition of the herbaceous community, affected soil chemistry. Irrigation increased  
33 K and P concentrations and  $\text{NH}_4^+$ -N availability. This study highlights the overall slow  
34 dynamics of soil chemistry in Mediterranean ecosystems, which **has resulted** in little  
35 variation of soil properties in reforested cropland after **more than a decade**.  
36 Reforestation can accelerate the recovery of some soil properties of abandoned cropland  
37 in comparison with secondary succession, but these effects will be more noticeable in  
38 longer time periods.

39  
40 **Key words:** **ammonium; inorganic nitrogen; nitrate;** old fields; *Quercus ilex*; soil  
41 fertility; tree planting.

42

43

## 44 **1. Introduction**

45           Large extents of cropland have been abandoned during the last decades due to a  
46 number of ecological and socio-economic factors (Bakker et al., 2005; Rey Benayas et  
47 al., 2007). Further, a considerable amount of cropland may be abandoned in the near  
48 future due to human migration from rural areas (Grau and Aide, 2008) or subsidies for  
49 cropland reforestation such as those from the EU Community Agrarian Policy or  
50 China's Grain to Green project (Cao et al., 2009). These areas can be left to undergo  
51 secondary succession (passive restoration) or be subjected to active restoration that  
52 mostly consists of tree and shrub planting and their management. **At present**, more  
53 abandoned agricultural land area is being restored by tree plantations than by secondary  
54 succession (FAO, 2011).

55           In semiarid ecosystems, recruitment of woody species is usually slow because it  
56 is hindered by factors such as extreme climatic conditions, poor soil fertility, and  
57 competition from herbaceous vegetation (Rey Benayas, 2005; Vallejo et al., 2006).  
58 Thus, Mediterranean abandoned cropland under secondary succession is initially  
59 colonized by herbaceous vegetation which persists for a long time before woody  
60 vegetation **establishes** (Bonet and Pausas, 2004). Restoration of these systems usually  
61 requires reforestation practices in order to reduce soil erosion, increase biological  
62 diversity and create carbon sinks.

63           There is evidence that reforestation not only alters **aboveground** vegetation, but  
64 also leads to significant changes in the physical and chemical properties and  
65 biochemical cycles of soils (Alriksson and Olsson, 1995; Côté et al., 2000; Paul et al.,  
66 2010b). After cropland abandonment, soil chemical properties may differ from natural  
67 soil properties due to previous agricultural practices such as fertilization, plowing, and  
68 harvesting (Koerner et al., 1997; Compton and Boone, 2000, Hooker and Compton,

69 2003). Generally, soil carbon increases with time after abandonment due to biomass  
70 accumulation, whereas available phosphorous decreases because of its immobilization  
71 in the living plant biomass (Du et al., 2007; Dölle and Schmidt, 2009). There is also a  
72 general consent that vegetation recovery can successfully restore soil nitrogen stocks  
73 (Alriksson and Olsson, 1995; Silver et al., 2005; Paul et al., 2010b). Since vegetation  
74 recovery differs between secondary succession and reforestation, soil chemical  
75 properties are expected to be different under these contrasting scenarios of  
76 Mediterranean woodland restoration.

77         Several studies have shown that plant species differ in their capacity to modify  
78 soil properties (Gallardo and Merino, 1993; Vinton and Burke, 1995; Cornelissen et al.,  
79 1999) since plant functional traits such as growth form, biomass allocation, tissue  
80 chemistry, and lifespan can significantly affect organic matter decomposition and  
81 nutrient dynamics (Hooper and Vitousek, 1998; Carrera et al., 2009). Plants with **fast**  
82 growth rates, such as herbaceous vegetation that usually proliferates on recently  
83 abandoned cropland, show high N concentration in green tissues and low content of  
84 secondary compounds which turn into high quality litter of **that decomposes rapidly**  
85 (Carrera et al., 2009). In addition, some plants such as the legumes have N-fixing  
86 capacity, which increases soil N content (Hooper and Vitousek, 1998; Oelmann et al.,  
87 2007; Davies et al., 2009). **In** contrast, plants with **slow** growth rates such as  
88 Mediterranean evergreen species exhibit high concentration of secondary compounds  
89 and chemical defenses against herbivores (e.g. phenol-protein complexes) **which**  
90 **promote slow** decomposition (Aerts and Chapin, 2000; Satti et al., 2003).

91         *Quercus ilex* (**Holm Oak**) is a slow-growing, sclerophyllous evergreen oak that is  
92 a major structural component of the natural woodlands in Western Europe and Northern  
93 Africa. **Holm Oak** produces large amounts of litter which can potentially incorporate

94 considerable quantities of organic matter and nutrients to the soil (Gallardo, 2003;  
95 Moreno et al., 2007). In addition, the micro-climatic conditions generated under tree  
96 canopies in dry climates, namely lower soil temperature and higher soil moisture,  
97 enhance microbial activity and increase litter decomposition and mineralization of  
98 organic matter (Muscolo et al., 2007; Sariyildiz, 2008). Thus, the presence of Holm  
99 Oak canopy may have both positive and negative effects on soil nutrient dynamics.

100 In this study, we analyze soil chemical properties associated with early secondary  
101 succession (hereafter referred to as old field) and reforestation with *Q. ilex* (hereafter  
102 referred to as reforested cropland) in a Mediterranean cropland that was abandoned 13  
103 years ago. As the response of soil to the environment is usually slow, most of this type  
104 of soil research is based on chronosequences or “space for time” studies (e.g. Alriksson  
105 and Olsson, 1995; Compton and Boone, 2000, Falkengren-Grerup et al.; 2006, Paul et  
106 al., 2010). However, we used an experimental approach rather than a phenomenological  
107 approach to test the effects of environmental manipulation on soil properties. Our study  
108 is part of long-term research aiming to investigate the effects of environmental  
109 manipulation on ecosystem processes in relation to restoration of Mediterranean  
110 woodland (Cayuela et al., 2008; Rey Benayas et al., 2008a). In this context, we also  
111 evaluated the relevance of previous management in addition to current environmental  
112 conditions on soil chemistry of the reforested woodland. Based on the contrasting  
113 effects of canopy of Holm Oaks on soil nutrient dynamics, we asked whether nutrient  
114 concentration and availability would be higher in the reforested woodland than in the  
115 old field or *vice-versa*. We hypothesized that current soil chemical properties of actively  
116 reforested woodland will reflect both a carry-over effect of previous management and a  
117 response to current environmental conditions. Most tree plantations in Mediterranean  
118 environments are based on pine species (MAPA, 2006), and little attention has been

119 given to how plantations **species** of native oak modify soil chemical properties. Our  
120 research will help to forecast effects of cropland reforestation on soil properties in  
121 comparison to secondary succession in Mediterranean environments, as well as offer an  
122 opportunity to test how previous environments resulting from reforestation management  
123 affect the current soil chemical properties.

124

## 125 **2. Materials and methods**

### 126 *2.1. Study site*

127 The study site was located in 1ha of abandoned cropland in central Spain  
128 (40°3'N, 4°24'W, altitude 450 m), which had been cultivated for grain for at least four  
129 decades until the experiment started. It has a typical Mediterranean continental climate,  
130 with mean annual precipitation of 480 mm and mean annual temperature of 15 °C.  
131 Summer is hot and dry while winter is cold with frequent frosts. The soil is a luvisol  
132 type derived from sandstone arkoses **and** is classified as loamy sand (62% sand, 23%  
133 silt, and 15% clay). **Before abandonment, the cropland was fertilized following a**  
134 **standard scheme in the area: application of a fertilizer with inorganic nitrogen,**  
135 **phosphorous and potassium (70:35:35, 400 kg/ha) once a year plus another annual**  
136 **application of just inorganic nitrogen (27% concentration, 150 kg/ha).**

137 In this abandoned cropland, we assessed two contrasted strategies of vegetation  
138 restoration, namely secondary succession and cropland reforestation. We assessed 20 10  
139 × 10 m-plots in total, **four** of which were under secondary succession (old field plots)  
140 and 16 were reforested (reforested cropland plots). The old field plots were located  
141 close to reforested cropland (<20 m apart). Soil parent material and depth were identical  
142 in all 20 plots, which were also subjected to identical soil preparation (plowing) before  
143 the reforestation took place in 1993. The 16 reforested plots were planted with 50 one-

144 year-old seedlings of *Quercus ilex* subsp. *ballota* planted at regular intervals of 2m (Rey  
145 Benayas, 1998). During the first three years, the planted seedlings were subjected to one  
146 of four treatments from the factorial combination of summer irrigation and artificial  
147 shading (control, irrigation, shading, and irrigation and shading) with four replicate  
148 plots per treatment. Irrigation was applied uniformly with sprinklers at the peak of the  
149 dry season (60 mm in July and August; 120 mm in total per year) and added across the  
150 whole plot area. The shading treatment consisted of a 68% reduction in incident  
151 radiation by placing a black polyethylene net 2 m above ground. The shading and  
152 irrigation treatments were stopped in the winter of 1996 and all plots have experienced  
153 natural rainfall and light conditions since then. The plots were protected from  
154 herbivores (sheep, rabbits and hares) with appropriate fencing.

155

## 156 2.2. *Characterization of vegetation and litter*

157 Variables related to vegetation structure and composition and plant litter were  
158 measured both in the old field and in the reforested cropland plots. In May 2006, five 50  
159 × 50 cm quadrats were established in each plot. In these quadrats, herbaceous  
160 aboveground biomass and herbaceous and Holm Oak litter mass were collected and  
161 weighed after drying at 60 °C for two days. All vascular plant species were identified  
162 and recorded in these five quadrats and in four additional quadrats. All species were  
163 classified into groups according to their functional attributes as graminoids, legumes  
164 and forbs (see Cayuela et al., 2008 for species composition details). We calculated the  
165 mean value of each measured variable (herb biomass, Holm Oak and herb litter mass,  
166 and total herbaceous, graminoid, legume and forb cover) per plot. In December 2005  
167 the volume of Holm Oak canopies in reforested cropland plots was calculated as the  
168 sum of the individual volumes, estimating the volume of each tree as its height × crown



169 projected area. The crown projected area of each **Holm Oak** was estimated as the  
170 elliptical surface of the crown projected onto the ground.

171

### 172 *2.3. Soil chemical properties*

173 Soil samples were taken from the four old field and 16 reforested cropland plots  
174 in March 2006. We systematically collected three 20 cm deep soil samples per plot at  
175 one of the plot diagonals; the location of the three sampling points divided the diagonal  
176 in four segments of similar length. These samples were combined into one single soil  
177 composite sample per plot (20 composite samples in total). Fresh soil composite  
178 samples were sieved to separate plant material and fragments >2 mm in size. **For** each  
179 composite sample, soil pH, carbon (C), total nitrogen (N), total phosphorous (P), and  
180 potassium (K) were measured. Soil pH was determined in a 1:2.5 mass:volume soil and  
181 water suspension. **Carbon** was analyzed using  $K_2Cr_2O_7$  in a  $H_2SO_4$  environment (Nelson  
182 and Sommers, 1982). Total N was determined by Kjeldahl analysis with  $SeSO_4-K_2SO_4$   
183 as catalyst in a Tecator 20 digestion system and a Kjeltex-auto 1030 analyzer (Tecator,  
184 Sweden). For total P, we used the method reported by Burriel and Hernando (1950).  
185 **Potassium** was analyzed according to MAPA (1986) using an Optic PLASMA ICP  
186 (Perkin- Elmer, model 4300 DV).

187 Potential rates of ammonification, nitrification and mineralization were  
188 determined by aerobic incubation of 5 g of dry soil of each composite sample with 15 g  
189 of pure sand and 6 ml of water for 14 days in the dark at 30 °C. Mineral N was extracted  
190 with 100 ml of KCl 2 N (soil:KCl 2 N ratio, 1:4), shaken for 1 h and the suspension  
191 filtered through 0.45 mm millipore filters. **Ammonium** ( $NH_4^+-N$ ) and **nitrate** ( $NO_3^- -N$ )  
192 in the extract were measured by colorimetry, using a microplate reader (Sims et al.,  
193 1995). Potential net mineralization rate was calculated as the difference between the

194  $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$  concentration before and after the incubation period. Potential net  
195 nitrification was the difference of  $\text{NO}_3^-\text{-N}$  concentration over the same period. Potential  
196 ammonification rate was the difference between potential net mineralization rate and  
197 potential nitrification rate.

198 In May 2006, the availability of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , total N ( $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$ )  
199 and phosphate ( $\text{PO}_4^{3-}\text{-P}$ ) in soils were assessed using anionic and cationic exchange  
200 membranes (types I-100 and I-200, Electropure Excellion, Laguna Hills, California).  
201 We used two anion and two cation exchange resin membranes that were placed at the  
202 two external sampling points of each plot diagonal and the mean value per plot was  
203 calculated. Resin membranes were previously conditioned in the lab by immersing them  
204 in demineralised water at 82–90 °C for 48 h. After conditioning, 2.5 ×2.5 cm resin  
205 membranes were glued on a plastic holder to facilitate insertion into the soil. A plastic  
206 rod joined to the plastic holder helped to locate the resin membranes in the field. This  
207 design kept the membrane ionic exchange capacity unaltered (Cain et al., 1999).  
208 Exchange resin membranes were introduced in the soil at a ca. 10 cm depth and  
209 remained in the soil for 20 days. After being removed, the membranes were dried at  
210 ambient temperature. The attached soil was removed, the plastic rod was cut and an  
211 extraction was performed with 50 ml of 2M KCl (5 cm<sup>2</sup> of resin membrane per 50 ml of  
212 2M KCl) by orbital spinning for 1 h at 200 rpm in 125 ml flasks. These extracts were  
213 used to calculate the quantity of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and total N-mineral by the  
214 indophenol blue method (Sims et al., 1995) and  $\text{PO}_4^{3-}\text{-P}$  by the molybdenum blue  
215 method (Allen et al., 1986) with a microplate reader.

216

217 *2.4. Effects of previous and current environmental conditions*

218 The management treatments (irrigation and shading) applied to the 16 reforested  
219 cropland plots between 1993 and 1995 were used to account for the environmental  
220 conditions during early establishment of the introduced *Q. ilex* seedlings.

221 We measured different variables to describe the effect of current environmental  
222 conditions in each reforested cropland plot. In addition to the volume of Holm Oak  
223 canopy, herbaceous **aboveground** biomass and litter mass, we estimated the canopy  
224 openness and soil moisture. Canopy openness was assessed by means of hemispherical  
225 (or fish-eye) digital photographs taken just before sunrise with a Nikon Coolpix 4500  
226 camera with a Nikon Fisheye Converter FC-E8 0.21x and analyzed with WinPhot 5.00  
227 software (Hans ter Steege, Utrecht University, 1996). We took photographs at nine  
228 sampling points per plot set with a regular distribution and the mean value per plot was  
229 calculated. Soil moisture in the first 10 cm was measured in each plot at five sampling  
230 points regularly **spaced** within the plot. We used a time domain reflectometer (TDR,  
231 Topp et al. 1980) on three dates (13 May, 23 May and 7 June 2006), and the mean value  
232 per plot was calculated. To account for the effect of current environmental conditions,  
233 we used volume of **Holm Oak** canopy, herbaceous mass, and composition of the  
234 herbaceous communities as explanatory variables. Across reforested cropland plots,  
235 volume of **Holm Oak** canopy was negatively correlated with canopy openness ( $r = -$   
236  $0.74$ ;  $p = 0.001$ ;  $n = 16$ ) and soil water content ( $r = -0.52$ ;  $p = 0.041$ ;  $n = 16$ ), and  
237 positively correlated with Holm Oak litter ( $r = 0.73$ ;  $p = 0.001$ ;  $n = 16$ ). Since these four  
238 variables were highly correlated, we only used canopy volume in the statistical  
239 analyses. In order to reduce the dimensionality of the species composition of the  
240 herbaceous community data set into one single variable, a non-metric multidimensional  
241 scaling (NMDS) was performed and the values for the first axis were selected as values  
242 of species composition.

243

## 244 2.5. Data analysis

245 Differences among soil chemical properties, vegetation cover and litter mass  
246 between old field and reforested cropland plots were analyzed with Student's t tests. For  
247 these analyses, we used only the control reforested plots, where irrigation and artificial  
248 shading treatments were not applied.

249 In the reforested cropland plots, differences in soil chemical properties,  
250 vegetation cover and litter mass among the four previous management treatments  
251 (control, irrigation, shading, and irrigation and shading) were analyzed by means of  
252 two-way ANOVA, in which irrigation and shading were the factors analyzed.

253 To test the simultaneous responses of soil properties to previous and current  
254 environmental conditions, the variance of soil properties was partitioned into different  
255 components by means of redundancy analysis (RDA) (Borcard et al., 1992). We  
256 differentiated direct effects of previous management, direct effects of current  
257 environmental conditions (i.e. Holm Oak canopy and herbaceous community), indirect  
258 effects of previous management through effects on current environmental conditions,  
259 and indirect effects of Holm Oak canopy through effects on the herbaceous community.  
260 The partitioning of the variance analysis allows estimation of the effects of each single  
261 variable or the effects of a group of variables; thus, the effects of the herbaceous  
262 community refer to both herb community composition and biomass. Indirect effects do  
263 not have degrees of freedom and, therefore, they cannot be tested for significance.

264 Data were checked for normality and homogeneity of variance, and were  
265 transformed when necessary to correct deviations from these assumptions. Differences  
266 between the levels of significant explanatory factors were determined using post-hoc

267 Tukey's tests. All statistical analyses were performed with Statistica 6.0. Package  
268 (StatSoft, Inc., Tulsa, OK, USA) and R 2.8 (R Development Core Team 2008).

269

### 270 **3. Results**

#### 271 *3.1. Vegetation structure and litter mass*

272 **Holm Oak** canopy was relatively closed in the reforested cropland (mean of  
273 56.1% in the four reforested control plots), whereas not a single **Holm Oak** was  
274 established in the old field plots after 13 years of cropland abandonment. **Consequently**,  
275 there was a relatively high quantity of **Holm Oak** litter in the reforested cropland (Table  
276 1), but not in the old field. The old field had higher herbaceous cover ( $62.1 \pm 4.6\%$ ),  
277 herbaceous biomass ( $143.9 \pm 12.3 \text{ g m}^{-2}$ ) and herbaceous litter ( $126.2 \pm 20.7 \text{ g m}^{-2}$ ) than  
278 the reforested cropland (Table 1;  $p = 0.035$ ,  $p = 0.024$  and  $p = 0.042$  for these variables,  
279 respectively). Leguminous ( $p = 0.113$ ) and graminoid ( $p = 0.661$ ) cover were similar in  
280 the old field ( $3.7 \pm 1.1\%$  and  $24.6 \pm 4.8\%$ , respectively) and in the reforested cropland  
281 (Table 1), but forb cover was higher in the old field ( $33.8 \pm 3.8\%$ ) than in the reforested  
282 cropland (Table 1,  $p = 0.048$ ).

283 Volume of **Holm Oak** canopy did not differ among reforested cropland plots  
284 subjected to previous management treatments (Table 1). However, canopy openness and  
285 **Holm Oak** litter in control plots were lower than in both irrigated plots and shaded plots  
286 (Table 1). Herbaceous biomass and cover were lower in shaded plots, but there were no  
287 differences in herbaceous litter among plots subjected to different management  
288 treatments (Table 1). Irrigation increased legume cover and shading reduced graminoid  
289 cover, but the treatments did not affect forb cover (Table 1).

290

#### 291 *3.2. Effects of vegetation restoration on soil chemical composition*

292 Soil pH was similar in the reforested cropland ( $5.71 \pm 0.22$ ) and in the old field  
293 ( $5.70 \pm 0.16$ ). Concentration of soil C (Fig. 1a) was 25% higher in the reforested  
294 cropland than in the old field. Neither, N, P or K concentrations in the soil differed  
295 between the reforested cropland and the old field (Fig. 1b, 1c and 1d). The C:N ratio of  
296 soil was similar in the reforested cropland ( $10.71 \pm 0.39$ ) and in the old field ( $10.37 \pm$   
297  $0.36$ ).

298 The concentration of soil  $\text{NH}_4^+$ -N was almost twice that in the reforested cropland  
299 than in the old field, whereas no differences were found for the concentrations of  $\text{NO}_3^-$ -  
300 N or total mineral N (Fig. 2a). Availability of  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N and total mineral N  
301 were 65-77% higher in the reforested cropland than in the old field (Fig. 2b). In both old  
302 field and reforested cropland, the availability of  $\text{NH}_4^+$ -N in the soil was higher than that  
303 of  $\text{NO}_3^-$ -N (78% and 67%, respectively). Soil ammonification, nitrification, and  
304 mineralization rates did not differ between reforested cropland and old field (Fig. 2c).  
305 Similarly, no differences were found for availability of  $\text{PO}_4^{3-}$ -P in the soil ( $6.06 \pm 0.41$   
306  $\mu\text{g dm}^{-2} \text{ day}^{-1}$  and  $6.39 \pm 1.28$ , respectively;  $p = 0.917$ ).

307

### 308 *3.3. Effects of previous and current environmental conditions on soil chemical* 309 *properties in reforested cropland*

310 Neither C, total N or the C:N ratio differed among soil collected from reforested  
311 cropland plots subjected to different previous management treatments. Soil K was the  
312 highest in irrigated plots (either shaded or not) and lowest in shaded plots, whereas  
313 control plots had intermediate values (Table 1). Irrigated plots showed the highest  
314 concentration of soil P, shaded and control plots the lowest, and the plots that were both  
315 irrigated and shaded had intermediate values. Concentrations of  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N and  
316 total mineral N in the soil did not differ among previous treatment plots. In contrast,

317 availability of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and total mineral N differed among treatments (Table  
318 1). **Soil from** irrigated plots and shaded plots had higher availability of  $\text{NH}_4^+\text{-N}$  than  
319 plots that were both irrigated and shaded, whereas control plots had intermediate values.  
320 Availability of  $\text{NO}_3^-\text{-N}$  and total mineral N were lower in plots that were both irrigated  
321 and shaded **compared to other** treatment plots. The four **management** treatments did not  
322 have any effect on availability of  $\text{PO}_4^{3-}\text{-P}$  **or** on potential rates of ammonification,  
323 nitrification and mineralization (Table 1). Soil water content was higher in irrigated  
324 plots than in the **other** treatment plots (Table 1).

325 Direct effects of previous treatments at **the time of** seedling establishment ( $p =$   
326  $0.032$ , D1 in Fig. 3) and of the herbaceous community ( $p = 0.499$ , D3) explained most  
327 of the variation in soil chemical properties in reforested cropland plots (15 and 20% of  
328 variance accounted for, respectively). **In contrast, the volume of the Holm Oak** canopy  
329 explained only 2% of the variance ( $p = 0.345$ , D2). Similarly, the indirect effects of  
330 **management** treatments **on** the herbaceous community (I2 in Fig. 3) explained 16% of  
331 the variance, whereas indirect effects **on** the **Holm Oak** canopy (I1) explained only 2%  
332 of the variance. **The effect of the Holm Oak** canopy on the herbaceous community (I3)  
333 indirectly explained 1% of the variance.

334

## 335 **4. Discussion**

### 336 *4.1. Secondary succession versus Holm Oak reforestation*

337 Our results showed that **only** few soil chemical properties, namely concentration  
338 of C and  $\text{NH}_4^+\text{-N}$  and availability of mineral N, differed between **two types of**  
339 **vegetation restoration possible for abandoned cropland regardless on their** clearly  
340 different vegetation structure (i.e. development of a dense **Holm Oak** canopy in the  
341 reforested plots). Thus, soil changes induced by agricultural practices may persist or

342 exhibit a legacy for a long time after cropland abandonment (Koerner et al., 1997;  
343 Compton and Boone, 2000). Other studies, particularly **those** in temperate prairies and  
344 forests, have reported restoration of soil fertility only after a few decades of cropland  
345 abandonment (Rey Benayas et al. 2009).

346 Thirteen years after cropland abandonment, the old field was colonized by a  
347 dense herbaceous community where no woody species **has** established. The recovery of  
348 woody vegetation in Mediterranean old fields is usually hindered by summer drought  
349 and herbaceous competition (Bonet and Pausas, 2004; Rey Benayas, 2005; Vallejo et  
350 al., 2006). Reforestation in this study created a closed **Holm Oak** canopy, which reduced  
351 herbaceous cover and biomass, probably because of water, nutrient and light deprivation  
352 (Ludwig et al., 2004; Pecot et al., 2007). Herbaceous community composition differed  
353 between old field and reforested plots, forb cover was higher in the **old field**.  
354 Differences in vegetation structure and composition between the old field and the  
355 reforested plots **are likely to** contribute to the differences observed in some of the  
356 studied soil chemical properties (Vinton and Burke, 1995; Cornelissen et al., 1999;  
357 Cornwell et al., 2008; Guo et al., 2008).

358 **Soil from** reforested cropland showed higher concentration of C than **soil from**  
359 old field **which is** in agreement with previous studies (Compton and Boone, 2000;  
360 Falkengren-Grerup et al., 2006). **Carbon** accumulation in soils under secondary  
361 succession after crop abandonment is slow in Mediterranean environments (Berg et al.,  
362 1993; Couteaux et al., 1995), **whereas** reforestation can accelerate the incorporation of  
363 C into the soil (Zhang et al. 2010).

364 Concentrations of total N, P and K, and pH did not differ between reforested  
365 cropland and old field plots. Some studies have reported increases in total soil N after  
366 agriculture abandonment and woody vegetation recovery (Alriksson and Olsson, 1995;



367 Côté et al., 2000), other studies have failed to detect such increases (Camill et al., 2004;  
368 Marcos et al., 2007). Agricultural land use increases the concentration of N, P and K in  
369 the soil due to application of fertilizers and this effect usually persists for long time after  
370 crop abandonment (Compton and Boone, 2000; Smal and Olszewska, 2008). It is likely  
371 that our experiment has not run a sufficient period of time to override such effects of  
372 agricultural fertilization.

373 Potential rates of mineralization were very low, or even negative, indicating a  
374 high potential of soil biota to immobilize N in our study (Gallardo and Merino, 1998).  
375 The lack of differences in ammonification, nitrification and mineralization rates  
376 between old field and reforested plots can again be explained by the relatively short  
377 time since cropland abandonment and that has low productivity environment. Changes  
378 in litter quality due to different plant composition may require a longer time to modify  
379 mineralization rates, since decomposition and incorporation of organic matter into the  
380 soil is usually slow (Berendse et al., 1989), particularly in Mediterranean environments  
381 (Berg et al., 1993; Couteaux et al., 1995). Moreover, mineralization rates were  
382 measured at standard environmental conditions in the laboratory and, consequently, the  
383 indirect effects of large plants on microclimatic conditions were omitted. Actual rates of  
384 mineralization in the field could well differ between old field and reforested plots.

385 Soils from old field and reforested cropland showed similar concentrations of  
386 total mineral N and  $\text{NO}_3^-$ -N. However, reforested plots displayed higher concentration  
387 of  $\text{NH}_4^+$ -N and higher availability of mineral N than old field plots. The amount of  
388 mineral N in the soil depends mainly on the balance between rates of mineralization and  
389 immobilization (Killham, 1994; Accoe et al., 2004). Since net N mineralization was  
390 similar in the old field and reforested plots, the lower amount of  $\text{NH}_4^+$ -N and the lower  
391 availability of mineral N found in the old field suggest that immobilization of N was

392 higher **here** than in reforested cropland. This means that N uptake by plants and /or soil  
393 microorganism exceeds the rate at which N is released through decomposition of  
394 organic matter. Fast growing species such as herbs are more nutrient demanding than  
395 slow growing **Holm Oak** and therefore have greater potential to uptake nutrients  
396 (Poorter et al., 1990), which may explain the lower concentration of  $\text{NH}_4^+$ -N in old  
397 field plots. Furthermore, previous studies demonstrated that N immobilization in the  
398 soil microbial biomass is higher in grassland than in forest soils (Davidson et al., 1990;  
399 Davidson et al., 1992; Hart et al., 1993), which can explain the **greater** availability of  
400 soil mineral N in reforested cropland. Differences in cover of **functional groups** in the  
401 herbaceous community between the old field and the reforested cropland may also  
402 explain differences in soil chemistry (Tilman et al. 1997). Legumes usually increase N  
403 availability in the soil, because of  $\text{N}_2$  fixation and higher N input via litter  
404 decomposition, whereas grasses and forbs reduce it, generally due to their high root  
405 production (Hooper and Vitousek, 1998; Oelmann et al., 2007; Davies et al., 2009).

406

#### 407 *4.2. Carry-over effects of reforestation management*

408 In agreement with our hypothesis, soil chemical properties in the studied  
409 reforested cropland reflected both carryover effects of previous reforestation  
410 management, particularly irrigation, and effects of current environmental conditions that  
411 were mostly related to herb community composition and biomass. This **pattern may** be  
412 explained by the mitigation of water stress on soil fauna, microbes and fungi that  
413 stimulate organic matter decomposition (Cousteaux et al., 1995; Austin et al., 2004).  
414 Soil chemical properties were also indirectly affected by previous management  
415 practices mainly through its influence on the herbaceous community, as indirect effects  
416 through **Holm Oak** canopy were low. The reforestation treatments applied determined

417 the structure and composition of the herb community (Cayuela et al., 2008), resulting in  
418 **differences in** litter quality and quantity which may have influenced soil properties  
419 (Vinton and Burke 1995). **For example, greater cover of legumes in irrigated plots** may  
420 explain the higher availability of N in these plots.

421 The relative effects of **Holm Oak** canopy were small compared to the effects of  
422 the herbaceous community. **Holm Oak** litter has a high lignin and tannin content (Allen  
423 et al., 1974), which results **in slower rates of** decomposition and **ultimately**  
424 incorporation of nutrients into the soil (Gallardo and Merino, 1993; Couteaux et al.,  
425 1995; Satti et al., 2003). The unexplained variation found in this study **may be due to** a  
426 combination of stochastic processes and mechanisms related to microclimate, **tree** root  
427 development **and soil biota** (Davidson et al., 1992; Couteaux et al., 1995; Aerts and  
428 Chapin, 2000).

429 To **complement** this research, it would have been desirable to study the soil  
430 chemical properties in nearby mature native woodland, **as** a reference ecosystem.  
431 However, **this was not possible as** there are no remaining patches of mature woodland in  
432 or near the study area.

433 This study provides further evidence of the overall slow dynamics of soil  
434 **processes** in Mediterranean ecosystems after cropland abandonment, even in reforested  
435 sites that are actively managed to facilitate the establishment of native woody  
436 vegetation. Yet experimental evidence allows us to highlight that reforestation with  
437 native woodland species may accelerate the recovery of soil properties such as the  
438 concentration of **C** and  $\text{NH}_4^+$ -N and the availability of mineral N, and **rehabilitation**  
439 **options** take advantage of management techniques used to facilitate early establishment  
440 of introduced seedlings. Thus, the **reintroduction** of woodland **into** agricultural

441 landscapes may contribute in the long term to enhance both biodiversity and ecosystem  
442 services linked to soil function (Rey Benayas et al. 2008b, Paul et al. 2010a and 2010b).

443

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658 **Table 1.** Vegetation cover, litter and soil variables measured in the reforested cropland  
659 plots under different treatments of previous management. Data are means  $\pm$  standard  
660 error. Different letters mean statistical differences at  $p \leq 0.05$ .

	Control	Irrigation	Shading	Irrigation and shading
<i>Vegetation cover and litter mass</i>				
Volume of Holm Oak canopy ( $\text{m}^3 \text{ha}^{-1}$ )	103.1 $\pm$ 41.1	209.8 $\pm$ 90.7	171.1 $\pm$ 42.1	137.8 $\pm$ 42.6
Holm Oak canopy openness (%)	68.5 $\pm$ 5.9 <sup>a</sup>	45.9 $\pm$ 3.4 <sup>b</sup>	45.4 $\pm$ 5.2 <sup>b</sup>	59.1 $\pm$ 3.9 <sup>ab</sup>
Holm Oak litter mass ( $\text{g m}^{-2}$ )	66.7 $\pm$ 24.0 <sup>b</sup>	127.5 $\pm$ 27.1 <sup>a</sup>	135.1 $\pm$ 7.7 <sup>a</sup>	83.1 $\pm$ 30.2 <sup>ab</sup>
Herbaceous biomass ( $\text{g m}^{-2}$ )	82.8 $\pm$ 15.6 <sup>a</sup>	54.9 $\pm$ 12.2 <sup>ab</sup>	35.1 $\pm$ 5.6 <sup>b</sup>	62.7 $\pm$ 18.4 <sup>ab</sup>
Herbaceous litter biomass ( $\text{g m}^{-2}$ )	78.1 $\pm$ 12.6	68.4 $\pm$ 11.8	74.5 $\pm$ 5.9	65.5 $\pm$ 11.1
Herbaceous cover (%)	58.2 $\pm$ 4.2 <sup>a</sup>	45.1 $\pm$ 4.7 <sup>ab</sup>	32.0 $\pm$ 3.6 <sup>b</sup>	51.0 $\pm$ 10.6 <sup>ab</sup>
Legume cover (%)	1.25 $\pm$ 0.8 <sup>bc</sup>	4.19 $\pm$ 1.1 <sup>ab</sup>	0.75 $\pm$ 0.2 <sup>c</sup>	4.71 $\pm$ 1.7 <sup>a</sup>
Graminoid cover (%)	24.3 $\pm$ 3.6 <sup>a</sup>	15.9 $\pm$ 1.2 <sup>ab</sup>	11.5 $\pm$ 2.0 <sup>b</sup>	14.1 $\pm$ 2.8 <sup>ab</sup>
Forb cover (%)	31.4 $\pm$ 2.7	25.0 $\pm$ 4.0	19.8 $\pm$ 2.9	24.8 $\pm$ 2.3
<i>Soil properties</i>				
pH	5.71 $\pm$ 0.22 <sup>b</sup>	6.49 $\pm$ 0.19 <sup>a</sup>	5.52 $\pm$ 0.05 <sup>b</sup>	6.55 $\pm$ 0.14 <sup>a</sup>
C concentration (%)	0.63 $\pm$ 0.02	0.53 $\pm$ 0.08	0.44 $\pm$ 0.06	0.55 $\pm$ 0.06
Total N concentration (%)	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04 $\pm$ 0.005	0.06 $\pm$ 0.01
C:N ratio	10.71 $\pm$ 0.39	10.60 $\pm$ 0.20	10.11 $\pm$ 0.40	9.70 $\pm$ 0.08
K concentration ( $\mu\text{g g}^{-1}$ )	172.3 $\pm$ 14.4 <sup>ab</sup>	256.8 $\pm$ 38.4 <sup>a</sup>	107.3 $\pm$ 6.29 <sup>b</sup>	230.4 $\pm$ 54.5 <sup>a</sup>
Total P concentration ( $\mu\text{g g}^{-1}$ )	15.81 $\pm$ 0.97 <sup>b</sup>	36.72 $\pm$ 8.15 <sup>a</sup>	14.30 $\pm$ 1.44 <sup>b</sup>	25.79 $\pm$ 5.92 <sup>ab</sup>
NH <sub>4</sub> <sup>+</sup> -N concentration ( $\mu\text{g g}^{-1}$ )	3.31 $\pm$ 0.24	1.88 $\pm$ 0.66	2.40 $\pm$ 0.43	2.83 $\pm$ 0.41
NO <sub>3</sub> <sup>-</sup> -N concentration ( $\mu\text{g g}^{-1}$ )	0.25 $\pm$ 0.49	0.47 $\pm$ 0.80	0.11 $\pm$ 0.87	0.78 $\pm$ 1.24
Total mineral N ( $\mu\text{g g}^{-1}$ )	3.56 $\pm$ 0.54	2.35 $\pm$ 1.43	2.51 $\pm$ 1.17	3.61 $\pm$ 1.49
NH <sub>4</sub> <sup>+</sup> -N availability ( $\mu\text{g dm}^{-2} \text{day}^{-1}$ )	19.1 $\pm$ 2.7 <sup>ab</sup>	24.3 $\pm$ 4.9 <sup>a</sup>	21.7 $\pm$ 1.0 <sup>a</sup>	10.7 $\pm$ 3.2 <sup>b</sup>
NO <sub>3</sub> <sup>-</sup> -N availability ( $\mu\text{g dm}^{-2} \text{day}^{-1}$ )	6.1 $\pm$ 0.4 <sup>a</sup>	6.1 $\pm$ 0.8 <sup>a</sup>	4.9 $\pm$ 1.4 <sup>a</sup>	1.9 $\pm$ 0.1 <sup>b</sup>
Total mineral N availability ( $\mu\text{g dm}^{-2} \text{day}^{-1}$ )	25.2 $\pm$ 3.2 <sup>a</sup>	30.4 $\pm$ 5.4 <sup>a</sup>	26.6 $\pm$ 2.2 <sup>a</sup>	12.5 $\pm$ 3.3 <sup>b</sup>
PO <sub>4</sub> <sup>3-</sup> -N availability ( $\mu\text{g dm}^{-2} \text{day}^{-1}$ )	6.1 $\pm$ 0.4	6.02 $\pm$ 0.8	7.5 $\pm$ 1.9	4.31 $\pm$ 0.3
Nitrification rate ( $\mu\text{g g}^{-1} \text{day}^{-1}$ )	0.30 $\pm$ 0.03	0.21 $\pm$ 0.10	0.23 $\pm$ 0.09	0.21 $\pm$ 0.08
Mineralization rate ( $\mu\text{g g}^{-1} \text{day}^{-1}$ )	0.22 $\pm$ 0.03	0.15 $\pm$ 0.09	0.23 $\pm$ 0.07	0.15 $\pm$ 0.08
Ammonification rate ( $\mu\text{g g}^{-1} \text{day}^{-1}$ )	-0.08 $\pm$ 0.01	-0.05 $\pm$ 0.01	-0.01 $\pm$ 0.06	-0.06 $\pm$ 0.01
Water content (%)	5.09 $\pm$ 0.86 <sup>b</sup>	7.70 $\pm$ 0.70 <sup>a</sup>	4.71 $\pm$ 0.41 <sup>b</sup>	5.97 $\pm$ 0.80 <sup>b</sup>

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662

663 **Fig. 1.** Soil C (a), total N (b), total P (c), and K (d) measured in the old field and  
664 reforested cropland plots. Data are means  $\pm$  standard error. \* mean statistical differences  
665 at  $p \leq 0.05$ .

666

667 **Fig. 2.** a) Concentration of soil ammonium, nitrate and total mineral N, b) availability of  
668 soil ammonium, nitrate and total mineral N, and c) rates of soil potential  
669 ammonification, nitrification and mineralization measured in the old field and reforested  
670 cropland plots. Data are means  $\pm$  standard error. \* mean statistical differences at  $p \leq$   
671 0.05.

672

673 **Fig. 3.** Contribution of direct effects (solid arrows) and indirect effects (dotted arrows)  
674 of previous and current environmental conditions to explain the variance (57.1%) of soil  
675 chemical properties in the reforested cropland plots (RC). D1: direct effects of previous  
676 management practices (control, irrigation, shading, and irrigation and shading); D2:  
677 direct effects of current Holm Oak canopy; D3 direct effects of current herb community  
678 (herb mass and species composition); I1 and I2: indirect effects of previous  
679 management through their effects on oak canopy and herb community, respectively; and  
680 I3: indirect effects of oak canopy through its effect on herb community.

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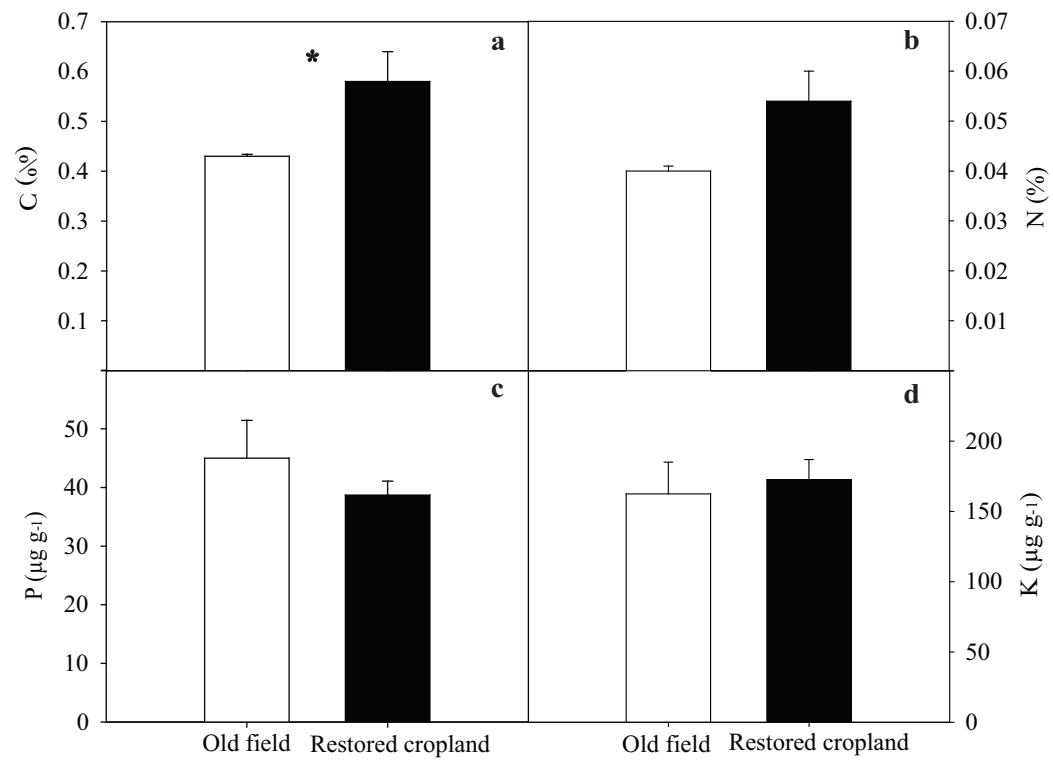
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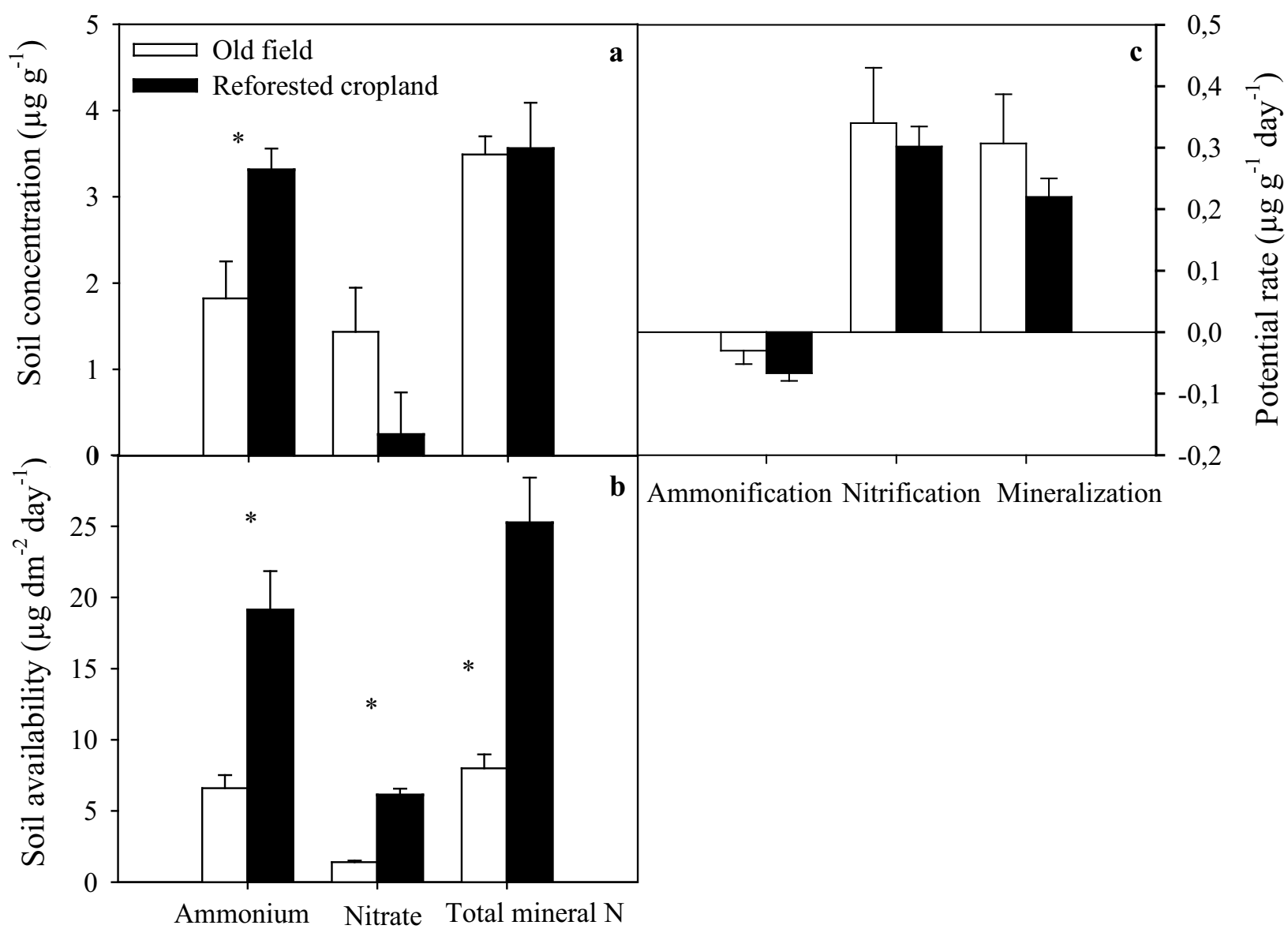
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Figure



Figure





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