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**Abstract**

This study assesses which factors are involved in the soil respiration (Sr) response to wetting-drying cycles in two Mediterranean ecosystems. We analysed Sr, mineral nitrogen, ion-exchange resin mineral nitrogen, and phosphate levels at weekly intervals over one year in two Mediterranean ecosystems with contrasting characteristics: a pine forest with high levels of organic matter and nutrients and a shrubland with low carbon and nutrients availability. Higher Sr was detected in the pine forest (0.12–0.76 g $CO_2$ $m^{-2}$ $hour^{-1}$) than in the shrubland (0.04–0.67 g $CO_2$ $m^{-2}$ $hour^{-1}$). For both sites, Sr increased during wet periods and decreased during dry periods. Compared with Sr in the pine forest, the trend observed for resin mineral nitrogen was the opposite. No pattern was observed for resin mineral nitrogen at the shrubland site, or for mineral nitrogen or phosphate at either site. The initial water status of the wetting-drying cycles determined the Sr response, whereas the length of the drought period before the rewetting event had no effect. The impact of the initial soil water content on Sr played a crucial role when the wetting-drying events occur in a dry soil, having a secondary role in wet soils. Finally, soil water status drove Sr during the growing season in both ecosystems; however, soil temperature had no effect on $CO_2$ efflux. In a changing world with projections of intensifying wetting-drying events, our results highlight the influence of soil water status on respiration rates, especially when these events occur in a dry soil.

**Keywords:** soil moisture; $CO_2$ efflux; mineral nitrogen; phosphate; resin mineral N; carbon mineralization

## 1. Introduction

Soil respiration involves the emission of $CO_2$ due to organic matter decomposition resulting from the metabolic processes of soil microbes and plant roots. Although the contribution of soil respiration is up to three-quarters of total ecosystem $CO_2$ effluxes into the atmosphere [1], we have only a limited understanding of the variability across ecosystems and the controlling factors [2]. In Mediterranean ecosystems, low soil moisture limits the response of soil carbon (C) and nitrogen (N) mineralization to increases in temperature [2, 3]. Climate models project longer dry periods and more intense rainfall events expected in these areas [4]. Given the key role of soil moisture in soil biogeochemical processes in these ecosystems, these projected climatic changes might cause long-term changes in soil C and N pools [5]. With the relatively limited stock of organic C in Mediterranean soils these ecosystems are particularly sensitive to climate change because any change in the precipitation pattern could alter soil respiration and potentially deplete stores of soil C.

Although Mediterranean ecosystems have been classically underestimated, the effects of soil temperature and moisture on both respiration rates [6] and N cycling [7] have been the focus of much research in the last decade in this area. These studies have provided valuable insights into the effects of wetting-drying cycles on soil processes, but many of them have produced in inconsistent findings (for a review, see Borken and Matzner [8]). Hence, more information is required to achieve a deeper understanding of the functioning of these ecosystems.

63 Studying the mineralization rates of soil C and N in Mediterranean ecosystems is
64 particularly challenging because of the difficulties entailed by the marked seasonality of
65 precipitation and the high spatial heterogeneity of soil properties and vegetation. This
66 heterogeneity is particularly acute in shrublands, in which the patchy distribution of
67 vegetation leads to increased localization of soil resources under shrub canopies [9].
68 Additionally, few studies have considered the effect of wetting-drying cycles on
69 phosphorous (P) cycling [10], although soil P might be highly limiting in terrestrial
70 ecosystems as a consequence of increased atmospheric N deposition [11]. Jenerette and
71 Chatterjee [12] reported that the microbial response to a wetting-drying event is
72 triggered by soil wetting but is regulated by resource limitation, demonstrating the
73 relevance of the soil nutritional status in these types of studies. We evaluated the
74 changes in soil respiration rates, mineral N (defined as the sum of ammonium and
75 nitrate), resin-mineral N, and $PO_4^{3-}$ during natural wetting-drying cycles in two
76 Mediterranean ecosystems distant few km between each other. The two study sites have
77 contrasting levels of soil organic matter and nutrients: a pine forest with high levels of
78 organic matter and soil nutrients, most likely the result of the dense plant-canopy
79 environment, and a less dense shrubland with low C and nutrient availability. A
80 comparison of these two spatially close but largely different ecosystems can provide
81 valuable information on how soil respiration is modulated by the local soil conditions.

82 Our goal was to assess which factors are involved in the soil respiration response
83 to wetting-drying cycles. We conducted an intensive weekly sampling for one year to
84 capture the entire intra-seasonal variability of the studied variables and to identify

85 different wetting-drying cycles within each season. This survey explored any emergent
86 and common patterns in soil respiration, N mineralization and mineral P responses to
87 these wetting-drying cycles. By considering whether the wetting events occurred in a
88 previously dry or wet soil, we attempted to assess the effect of additional rewetting on
89 the dynamics of the variables as a function of prior soil moisture. We also assessed the
90 effect of the length of the drying events before rewetting because it may play a key role
91 in the response of soil respiration to sudden changes in soil moisture, which may be
92 further modulated by both soil organic matter and soil texture [6, 13]. Experimental set-
93 up of this type gains importance in ecosystems with such features as high spatial
94 heterogeneity of soil properties and vegetation similar to that observed in semiarid
95 areas.

96 We hypothesized that (i) given the major differences between the two study
97 sites, the responses of soil respiration rate and mineral N and P to the wetting-induced
98 pulses would produce distinct patterns in the two Mediterranean ecosystems; (ii) soil
99 respiration would be driven by soil water content and temperature at the two study sites,
100 and (iii) both the length and severity of a drought period before rewetting would
101 determine the response of soil respiration to rapid changes in the soil water status.

## 2. Materials and Methods

### 2.1. Study area

This study was conducted in two ecosystems, pine forest and shrubland, in southwestern Spain (37° 21'N; 5° 56' W), both with a typical Mediterranean climate. The distance between the study sites is 14.5 km. The 30-year average rainfall and temperature at the experimental sites were 565.7 mm and 19.0 °C, respectively. The study year (October 2009 – October 2010) was wetter than normal, with rainfalls of 852.6 mm in the pine forest and 845.7 mm in the shrubland. The soils in these areas have a typical A(B)C profile with a sandy clay loam and loamy sand texture in the pine forest and the shrubland respectively, as defined by the United States Department of Agriculture [14]. Table 1 presents the primary properties of these soils. The pine forest is composed primarily of *Pinus pinea* L. with scarce annual herbs and forbs in the understory. The shrubland is dominated by *Quercus coccifera* L., *Cistus albidus* L., *Genista hirsuta* Vahl., and *Arbutus unedo* L.

### 2.2. Soil sampling

To explore the temporal dynamics of soil respiration and nutrient availability, we conducted soil sampling at weekly intervals over the course of one year (October 2009 to October 2010). Soil sampling was performed each Friday for the pine forest and each Saturday for the shrubland. The sampling areas were approximately 4000 and 3000 $m^2$ for the pine forest and shrubland, respectively. Each site was considered a single plot, and a minimum distance of 3 m between both soil samples and respiration

123 measurements and between measurements performed in different days was used to
124 ensure that pseudoreplication was avoided. Six soil samples from each study site were
125 randomly collected to a depth of 10 cm of the soil profile on each sampling date with a
126 circular soil corer (5 cm diameter × 10 cm height). We removed the litter layer from the
127 topsoil before sampling and then transported the samples in refrigerated plastic bags to
128 the laboratory for storage at 3 °C. The soil samples were sieved to remove roots and
129 rocks, processed within three days of collection, and subsequently analysed separately.
130 On each sampling date at six randomly chosen spots located on the bare soil, soil
131 respiration rates were measured as the surface $CO_2$ efflux using a portable soil
132 respiration system (EGM-4, PP SYSTEMS) with a chamber 10 cm in diameter and 15.5
133 cm in height. According to the manufacturer's protocol, the chamber was held in the air
134 to flush it out before each measurement and then placed on the soil for determination of
135 soil respiration rates. Soil temperature was monitored via a digital soil thermometer at
136 six randomly chosen spots different from those at which the soil samples were
137 collected. Both the soil respiration and temperature were systematically measured from
138 10:00 to 11:00 a.m. (local time, GMT+1) and sampled at different spots on each
139 sampling date after removing the litter layer.

140 **2.3. Laboratory analyses**

141 Soil texture was estimated using the hydrometer method proposed by Kroetsch
142 and Wang [15]. Soil pH was measured using a 1:5 soil-water solution ratio. Soil organic
143 matter was analysed following the wet oxidation techniques of Skjemstad and Baldock
144 [16]. Total soil N was measured following standard Kjeldahl procedures [17]. Dissolved

145 organic N (DON) was analysed following Sollins et al. [18]. DON contents were
146 estimated as the difference between total dissolved N and mineral N. Microbial
147 biomass-N (MB-N) was determined with the fumigation-extraction method as proposed
148 by Brookes et al. [19]. The MB-N concentration was estimated as the difference
149 between total N in the fumigated and unfumigated digested extracts divided by a Kn
150 (the fraction of MB-N extracted after $CHCl_3$ treatment) of 0.54 [19]. Soil phenols,
151 hexoses and aromatic compounds were determined following Chantigny et al. [20]. Mg,
152 K, Ca and Na were determined by atomic absorption spectrophotometry. All these
153 variables were analysed to generate a general soil description of the two study sites.

154 The following analyses were used to characterize the soil samples collected
155 simultaneously with each soil respiration sampling. Gravimetric soil moisture was
156 calculated for fresh 5 g subsamples after drying them at 80 °C for 48 h to reach a
157 constant weight. Soil water-holding capacity (WHC, g $H_2O$ 100 $g^{-1}$ soil) was
158 determined for each experimental site from the gravimetric water content of each soil
159 sample that was saturated and allowed to drain freely over 48 h into a filter funnel
160 covered with a plastic wrap at 4 °C [7]. To measure mineral N, soil subsamples were
161 extracted using 0.5 M $K_2SO_4$ at a ratio of 1:5. The soil samples were shaken with the
162 extractant in an orbital shaker at 200 rpm for 1 h at 20 °C and filtered through a 0.45 μm
163 Millipore filter. The filtered extract was stored at 2 °C until the colorimetric analyses
164 were conducted within seven days of extraction. The $NH_4^+$-N concentration was directly
165 estimated using the indophenol blue method with a microplate reader (ASYS Jupiter
166 HD-ASYS, Hitech, [21]). $NO_3^-$-N was first reduced to $NH_4^+$ with Devarda alloy, and the

concentration was determined as described above. The $NO_3^-$-N concentration in the extracts was estimated as the difference between the values of the Devarda-incubated and unincubated samples. Mineral N was expressed as the sum of $NH_4^+$ and $NO_3^-$.

The mineral N availability was measured *in situ* using ion-exchange membranes (resins [22]). We selected this technique because it generates minimal disturbances in soil surface communities and allows intensive sampling over multiple time periods at the same spatial location. Six anion and cation resins (types I-100 and I-200, Electropure Excellion, Laguna Hills, California) were installed per site per week over the one-year sampling period. Before installation, the resins were first subjected to expansion treatment by submersion in distilled water at 82–90 °C for 48 h. The resins were then cut into 2.5 × 2.5 cm squares, attached to a plastic rod with acrylic glue and inserted into the soil at a depth from 0.5 to 3 cm. Resins were incubated in the field for seven days during each sampling period. Following collection, the resins were dried at ambient temperature in the laboratory. Next, the resins were carefully separated from the plastic rod, brushed to remove soil particles, and placed into 125 ml flasks for extraction with 25 ml of 2 M KCl via orbital spinning (1 h at 200 rpm). The extracts were analysed to measure the mineral N contents using the above-mentioned methods [19]. To measure $PO_4^{3-}$-P, soil subsamples were extracted with 100 ml of 0.5 M $NaHCO_3$ at a ratio of 1:20, and the concentration in the extract was determined using the molybdenum blue colorimetric method [23].

### 2.4. Statistical and numerical analyses

During the study year, we identified eight major intervals of increasing soil water content which we defined as wetting events, and eight periods of decreasing soil water content defined as drying events, at each study site (Figure 1a). We found these intervals in all seasons, with lengths ranging from 1 to 9 weeks. We classified these intervals into dry or wet soil occurrences to calculate the effect of further wetting events on the variable dynamics in an already wetted or a relatively dry soil. We considered a wetting interval to occur on a previously wet soil when the initial soil moisture in % of the WHC was above 20% in both sites. A drying interval was considered to occur within a dry soil when the initial soil moisture in % of the WHC (i.e., immediately before the start of the drying interval) was below 40% in the pine forest or 50% in the shrubland. These thresholds were arbitrarily chosen as representative of previously wet and dry soils, respectively, in view of the results of soil moisture in % of the WHC obtained in Figure 1. For the starting point of the wetting event, we distinguished between sites because of the large difference between the soil water-holding capacities (45.68 g $H_2O$ 100 $g^{-1}$ soil for pine forest and 27.73 g $H_2O$ 100 $g^{-1}$ soil for shrubland; Table 1). Thus, we attempted to compensate for this difference and identify eight comparable wetting-drying cycles at the two study sites. The increases or decreases in the response variables were calculated as the difference between the final and initial concentrations in each wetting or drying interval. We estimated the Spearman correlation of these changes in soil variables during wetting-drying cycles with the mean soil temperature, mean soil water content, length of the wetting-drying cycles, and length of the prior wetting-drying cycle. We accounted for the length of the prior wetting-drying cycle by considering the number of weeks that the wetting or drying

event previous to the studied cycle lasted. Daily precipitation values were obtained from the AEMET weather station network near the study sites. Pearson correlation coefficients were calculated to test the effect of soil temperature and moisture on the $CO_2$ efflux. Spearman correlation coefficients were used to assess the effect of the lengths of wetting-drying cycles and prior wetting-drying cycle on soil respiration depending on soil moisture conditions.

To assess the effect of study site and season on the soil variables analysed, we used a linear mixed model, in which the study site was treated as a fixed effect and time was treated as a random effect. Our experimental design accounted for spatially independent samples within each site and considered time as a random effect using linear mixed models, which are particularly useful in settings that require repeated measurements. The effect of each independent variable on the model was analysed with a permutation test (1000 permutations of raw data). The linear mixed model and permutation tests were conducted using the "nlme" and "pgirmess" libraries, respectively, in the R statistical package, version 2.15 (R Development Core Team, 2012). The effects of the wetting-drying cycles on the changes in the analysed variables for each site were determined with a linear model and permutation tests, as described above, with a significance level of $P<0.05$.

## 3. Results

### 3.1. Intra-seasonal and annual variability

Soil respiration rates in the pine forest were higher than those in the shrubland ($P<0.001$, Table 1). The mean values for each sampling date ranged between 0.12 and 0.76 g $CO_2$ $m^{-2}$ $h^{-1}$ for the pine forest, and between 0.04 and 0.67 g $CO_2$ $m^{-2}$ $h^{-1}$ for the shrubland (Figure 2). We detected significant differences among the seasons ($P<0.001$, Figure 2), with a significant interaction between site and season ($P<0.001$). Differences among seasons were found during a separate site analysis ($P_{Shrubland}=0.001$, $P_{Pine\ Forest}<0.001$). The pattern of soil respiration rates at the two study sites was different throughout the seasons. Whereas soil respiration tended to increase from autumn to winter in the pine forest, the opposite trend was observed in the shrubland (Figure 2). In the pine forest, soil respiration tended to decrease from spring to summer, whereas no trend was observed for the shrubland in this period (Figure 2).

Mineral N values were significantly higher in the pine forest than in the shrubland ($P<0.001$, Table 1), with a significant interaction between site and season ($P<0.015$). The mean values for each sampling date ranged from below the detection limit to 13.48 mg $kg^{-1}$ soil for the pine forest and from below the detection limit to 11.13 mg $kg^{-1}$ soil for the shrubland (Figure 3a). Seasonal differences were found during a separate site analysis ($P_{Shrubland}=0.009$, $P_{Pine\ Forest}=0.009$). For both study sites, the maximum mineral N values occurred in autumn and summer, and the minimum values occurred in spring (Figure 3a). The pattern for mineral N content was similar at

250 both study sites, which tended to increase in autumn and summer and decrease in winter
251 and spring (Figure 3a).
252 We detected significant differences in the resin mineral N values among seasons,
253 but not between sites ($P_{season}<0.001$, $P_{site}=0.068$, Table 1). The maximum and minimum
254 mean values of resin mineral N were found in autumn and spring, respectively, at both
255 study sites, with values ranging from 0.11 to 2.45 μg $cm^{-2}$ $day^{-1}$ for the pine forest and
256 0.12 to 2.31 μg $cm^{-2}$ $day^{-1}$ for the shrubland (Figure 3b). Similar to the mineral N
257 values, the pattern for the resin mineral N content was similar at both study sites,
258 tending to increase in autumn and summer and decrease in winter and spring (Figure
259 3b). We found a significant interaction between site and season (P =0.005). Seasonal
260 differences were detected via a separate site analysis ($P_{Shrubland}<0.001$, $P_{Pine\ Forest}<0.001$).
261 The $PO_4^{3-}$ concentration was significantly higher in the pine forest (mean values
262 for each sampling date ranging from 0.33 to 7.00 mg $kg^{-1}$ soil, with an annual mean of
263 2.37 mg $kg^{-1}$ soil) than in the shrubland (mean values between 0.07 and 2.16 mg $kg^{-1}$
264 soil, annual mean of 0.53 mg $kg^{-1}$ soil; Table 1, Figure 3c). Whereas the common
265 pattern in the two study sites tended to remain steady from autumn to spring and
266 increase in summer (Figure 3c), we detected a maximum peak in early spring in the
267 shrubland (Figure 3c). Although we did not observe significant differences among the
268 seasons, the significant interaction detected via statistical analyses indicated that the
269 seasons influenced the differences between the sites ($P_{season}=0.471$, $P_{site\ x\ season}<0.001$).
270 Seasonal differences were detected in a separate site analysis for both study sites
271 ($P_{Shrubland}=0.007$, $P_{Pine\ Forest}=0.007$).

### 3.2. Changes during wetting-drying cycles

For both the annual average and in dry soils, soil respiration increased during wetting events and decreased during drying events in both the pine forest and shrubland (Table S1, Figure 4). However, soil respiration was not significantly different when wetting-drying cycles occurred in wet soils (Table S1, Figure 4). While an inverse pattern was found for resin mineral N at the pine forest, these differences were only significant for the annual average (Table S1, Figure 5). We found no pattern for resin mineral N in the shrubland (Table S1, Figure 5). Additionally, no trend was observed for mineral N or $PO_4^{3-}$ in response to wetting-drying cycles in any condition at either site (Table S1, Figure 5).

### 3.3. Influence of event duration, soil water content and temperature on respiration

Changes in soil respiration during wetting-drying cycles did not show any significant correlations with the lengths of the wetting-drying cycles or the prior wetting-drying cycle (Table S2). Pearson correlation revealed a significant effect of soil water content on the $CO_2$ efflux in both autumn and spring in the pine forest and in both spring and summer in the shrubland (Table S3). However, no significant effect of soil temperature on respiration rates was found for any season at either study site (Table S3).

290 ## 4. Discussion

291 Although we predicted a different pattern in the two ecosystems modulated by
292 local soil conditions, a similar soil respiration response was observed in the wet and dry
293 phases at both study sites. The two ecosystems have different levels of soil organic
294 matter and different soil texture (Table 1), but these differences were not sufficient to
295 trigger a difference in the microbial and plant root response to wetting-drying cycles.
296 Moreover, we found that the response of soil respiration was not significant at either site
297 when the wetting-drying cycles occurred on previously wet soil.

298 The length and severity of the drought period before a rewetting event might
299 play a key role in the response of soil respiration to the rapid changes in soil water
300 status that can occur in Mediterranean ecosystems [6]. In this study, we analysed the
301 effects of both factors in regulating the respiratory pulse, and the relative change in soil
302 water content likely had a critical effect on the soil respiration response. We found no
303 effect of the length of the drought period before the rewetting event or of the wetting or
304 drying events. We hypothesize that the magnitude of the changes in soil water potential
305 that occurred in an already-wet soil in response to wetting events was too small to cause
306 a rapid soil respiration response. Likewise, drying events that occurred in wet soil were
307 not sufficiently strong to reduce the $CO_2$ efflux because the soil remained wet after the
308 corresponding event. Thus, the impact of the initial water status on soil respiration is
309 crucial when the wetting-drying events occur in a dry soil, having a secondary role in
310 wet soils. Orchard and Cook [24] found a linear dependence of soil respiration on the
311 extent of change in water potential when a dry soil is wetted. Similarly, Fischer [25]

demonstrated that a rapid soil respiration response in a dry soil depends on the magnitude of the soil water potential change during rewetting. Our results are consistent with previous studies in Mediterranean ecosystems, showing that the respiratory pulse was stronger in summer than in spring when responding to a similar sized rewetting [6]. However, we found no effect of soil organic matter or texture on the response of soil respiration to moisture pulses; this finding is inconsistent with other studies performed in water-limited ecosystems [6, 13].

Several mechanisms may explain the changes in soil respiration rates detected in response to the wetting-drying cycles at the two study sites. Soil respiration pulses are generally derived from the microbial consumption of soil organic matter, and these pulses are highly responsive to precipitation pulses. The decomposable organic substrates are derived partially from the death of a portion of soil microorganisms and partially from the non-living soil organic matter [26]. When soil is rewetted, the surviving microorganisms readily decompose the portion of microbial biomass that dies under dry conditions. The rapid changes in soil water potential associated with rewetting may also cause microbes to undergo osmotic shock, leading to the lysis of microbial cells or the release of intracellular solutes [27]. Wetting-drying cycles also increase the availability of nonliving soil organic matter for decomposition via physical disruption of soil structure, substrate desorption from surfaces, and increases in microbial mobility and diffusion of soluble organic compounds [28].

Of note, the soil respiration measured in this study was an integrated measure of heterotrophic and autotrophic respiration. Thus, the role of plant root respiration must be considered, which is a large contributor to soil $CO_2$ efflux. Productivity through the

translocation of assimilated C to the rhizosphere is one of the main drivers of autotrophic soil respiration [29]. The net primary production in these pine forests is generally higher than that in the shrublands. Thus, the differences in ecosystem productivity may play a key role in the significantly higher soil respiration rates found in the pine forest than in the shrubland. Likewise, the higher soil organic matter levels found in the pine forest might foster biological activity, resulting in the higher soil $CO_2$ emissions at this site. Previous studies reported that C and N mineralization rates during wetting-drying cycles generally correlate with the soil organic matter content [30]. The respiration rates in the shrubland were much lower than those in the pine forest in autumn and winter, whereas the opposite trend was found in spring and summer. We attributed this result to site differences in soil properties, water availability, productivity, and plant community phenology [31]. Thus, although the differences in local conditions between the two study sites were not sufficiently strong to trigger contrasting respiration responses towards wetting-drying cycles, they could produce respiration rates of different magnitude and distinct seasonal trends for each site.

Interestingly, the upward trend in soil respiration during the growing season in the shrubland continued during the dry season (Figure 2). By contrast, during winter when the soil water content was above 15%, the response of soil $CO_2$ efflux to increases in soil moisture in the shrubland community was a linear decline (Figures 1 and 2).This unexpected pattern of increases of $CO_2$ rates in the rainless season and decreases in the wet season could be affected by the combination of a number of non-biological processes [32]. On one hand, the reduced soil $CO_2$ efflux observed in winter could be

357 due to soil saturated conditions impeding gas diffusion. The increased $CO_2$ rates found
358 in summer could reflect pedochemical and geological processes, such as geological
359 vents of $CO_2$ [33]. A complementary process could be the photochemical degradation of
360 litter by solar radiation during summer [34]. Other authors have recently reported
361 anomalous $CO_2$ rates in drylands that cannot be explained by biological processes [35,
362 36, 37].

363 Both geophysical processes and plant and microbial uptake influence the
364 concentrations of soil N and C, although soil moisture determines their
365 mineralization/immobilization. As shown in Figures 4 and 5, soil respiration in the pine
366 forest increased during wetting events and decreased during drying events, whereas the
367 opposite trend was observed for the availability of mineral N. We attributed this
368 difference in C and N mineralization under different soil water conditions to the fact
369 that soil respiration is generally enhanced by soil moisture through the above-mentioned
370 mechanisms, but belowground mineral N content is also highly dependent on specific
371 hydrological processes such as leaching and runoff [31]. Notably, the inorganic N pool
372 consisted almost entirely of $NO_3^-$-N in the two study sites (Table 1). Soil $NO_3^-$-N loss
373 through leaching and runoff is very common [38]. Our results suggest that these
374 processes might lead to N losses during wetting events, following an accumulation of N
375 during drying events at the pine forest site. By contrast, we did not detect changes in N
376 mineralization as a function of moisture in the shrubland. The higher values of
377 microbial biomass N found in the pine forest suggest potentially greater N

immobilization during organic matter decomposition compared with that in the shrubland soil.

The notably higher values of $PO_4^{3-}$ found in the pine forest than in the shrubland can be explained by the combination of four complementary mechanisms based on the different pH values, textures and soil organic matter characterizing the study sites (Table 1). First, in acidic soils such as that in the shrubland, Al reacts with $PO_4^{3-}$, which is gradually transformed into insoluble compounds of phosphate that become generally not available for microbes or plants [39, 40]. Second, the $PO_4^{3-}$ holding capacity of fine-textured soils is higher than that of coarse-textured soils, which hold less $PO_4^{3-}$ because of the more inert character of sand particles compared with that of clay particles [41]. Third, the soil $PO_4^{3-}$ in the shrubland may have been lost through soil erosion because this area is subjected to intense trampling [42]. Fourth, soils poor in organic matter such as that in the Shrubland could lead to less organic P mineralization [42].

Over the course of the study year, soil moisture drove the $CO_2$ efflux in autumn and spring in the pine forest and in spring and summer in the shrubland, whereas soil temperature did not determine the soil respiration in any season at either site. This result was in contrast to our hypothesis that both soil moisture and temperature would drive the soil $CO_2$ efflux. Soil respiration being mainly driven by moisture in water-limited ecosystems has been previously reported [3]. This reinforces the idea that soil temperature might be a secondary controller of $CO_2$ efflux in this type of ecosystem. However, other studies found that the seasonal pattern of soil respiration closely followed soil temperature in Mediterranean ecosystems [43]. These inconsistent results

400 indicate that the influence of soil temperature on $CO_2$ efflux might be variable
401 depending on factors such as ecosystem type, vegetation, and soil physicochemical
402 characteristics.
403

404 ## 5. Conclusions

405 The results presented in this study demonstrated that the response of soil
406 respiration to wetting-drying cycles was similar in the pine forest and the shrubland;
407 rates increased during wet events and decreased during dry events. However, we found
408 higher soil respiration rates in the pine forest (0.12–0.76 g $CO_2$ $m^{-2}$ $hour^{-1}$) than in the
409 shrubland (0.04–0.67 g $CO_2$ $m^{-2}$ $hour^{-1}$), and significant differences between the annual
410 trends in soil respiration at the two sites, which we attributed to different vegetation and
411 soil characteristics. Furthermore, our findings suggest that while the initial water status
412 of the wetting or drying events had a critical effect on the soil respiration response, the
413 length of the drought period before the rewetting event had no effect. We also
414 concluded that soil water status determined the $CO_2$ efflux during the growing season at
415 both sites, whereas soil temperature might play a secondary role in controlling soil
416 respiration in these types of ecosystems. More research is required to elucidate the
417 effect of wetting-drying cycles on soil respiration in a changing world in which these
418 episodes are projected to intensify.
419

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## Figure captions

**Figure 1.** (a) Evolution of soil moisture in % of the water holding capacity (WHC) measured at weekly intervals in the top 10 cm of the soil profile during the study year in the pine forest and shrubland sites. Grey bands indicate the eight selected wetting phases. White bands indicate the eight selected drying phases. Error bars are ± SE, N=6. (b) Soil temperature and precipitation at the pine forest and shrubland throughout the study year.

**Figure 2.** Absolute values of soil respiration rates measured at weekly intervals during the study period in the pine forest and shrubland. Shaded bands correspond to the selected wetting phases defined in Figure 1. Error bars are ± SE, N=6.

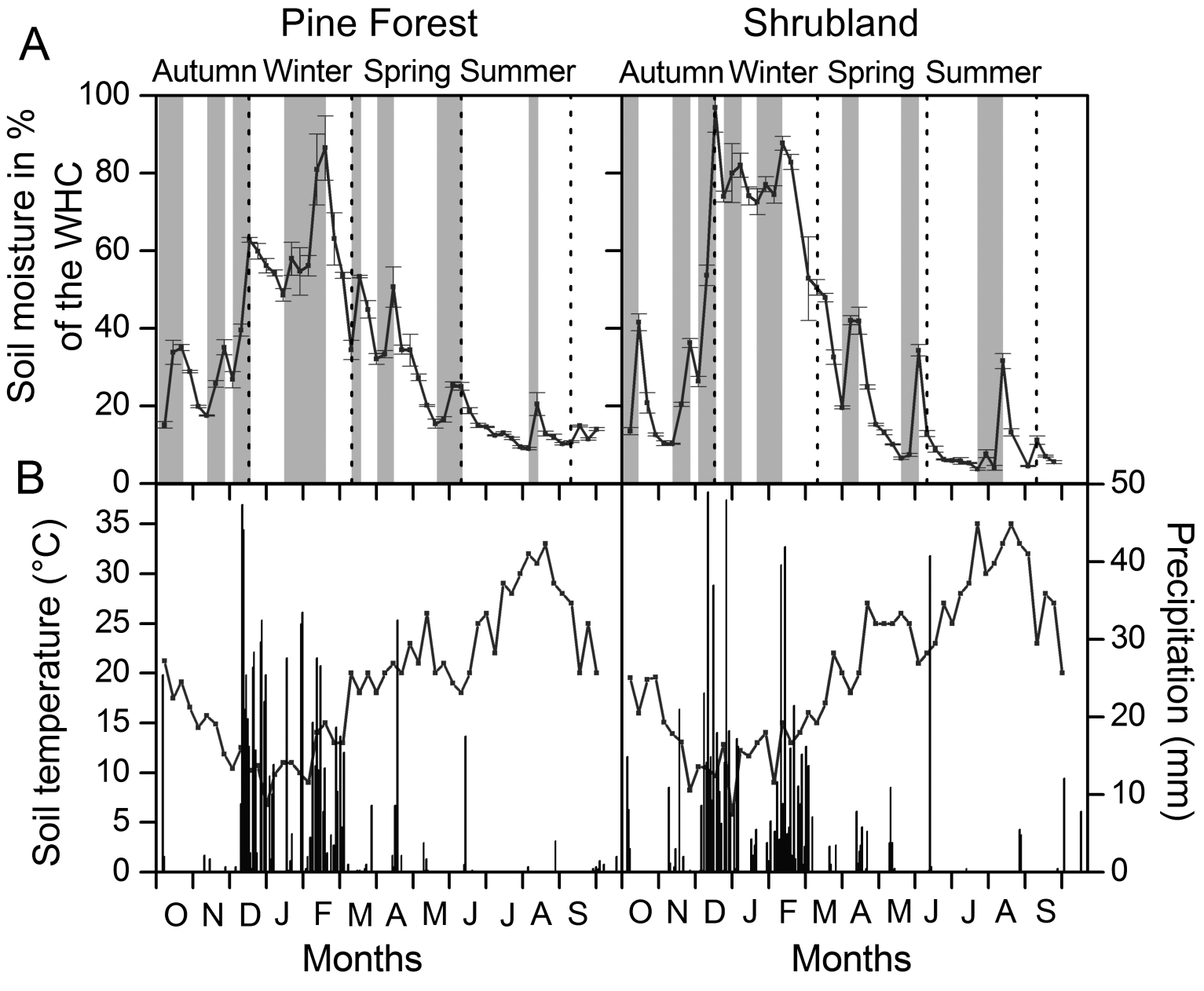
**Figure 3.** Absolute values of (a) mineral N, (b) resin mineral N, and (c) $PO_4^{3-}$ -P measured at weekly intervals throughout the study year in the pine forest and shrubland plant communities. Error bars are ± SE, N=6.

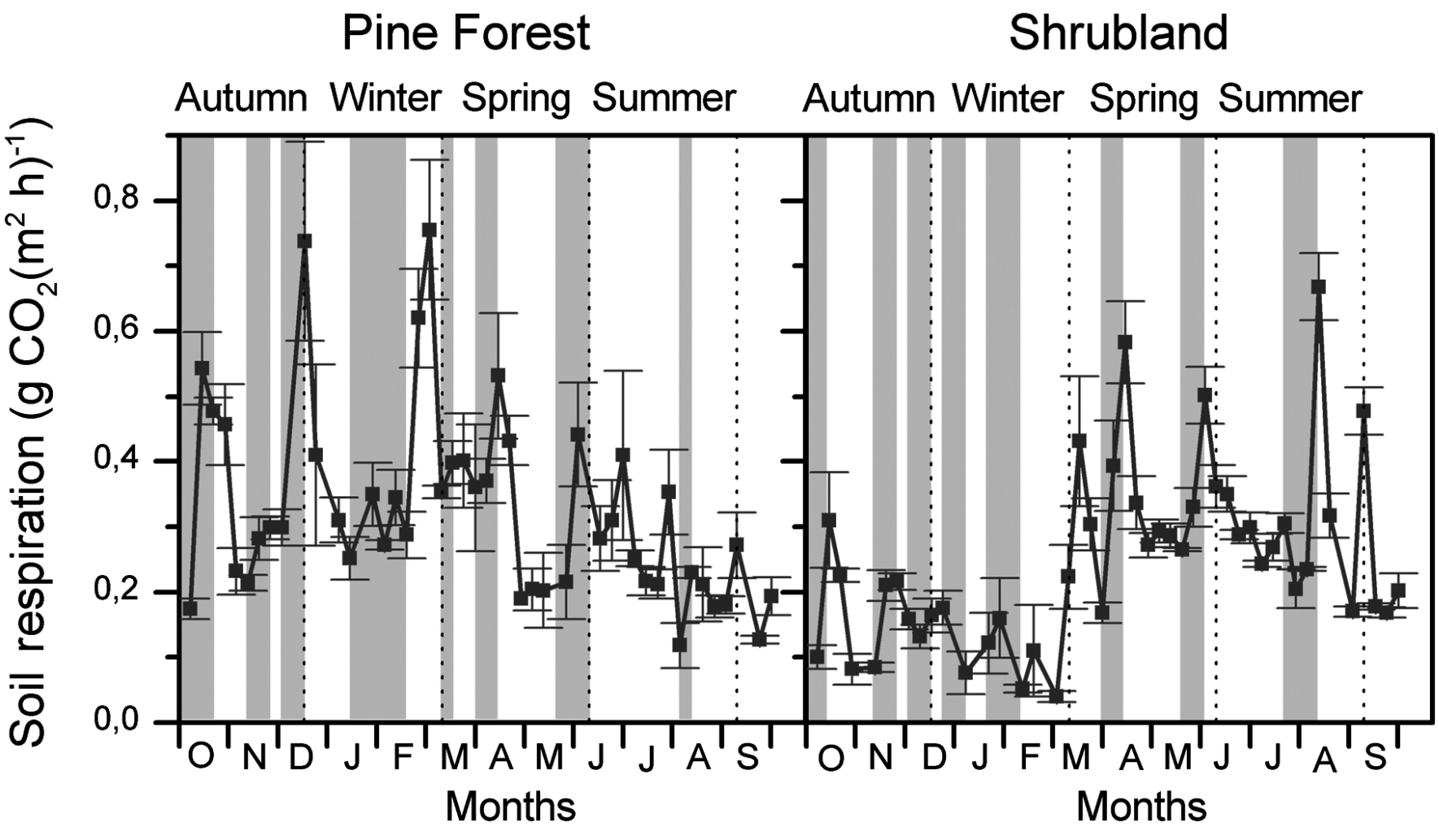
**Figure 4.** Changes of soil respiration rate during wetting-drying cycles at each study site. Data were analysed for the entire year and separately for dry and wet soils. Error bars are ± SE, with N=8 for the annual average and N=4 for dry and wet soils. Significant differences are indicated as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

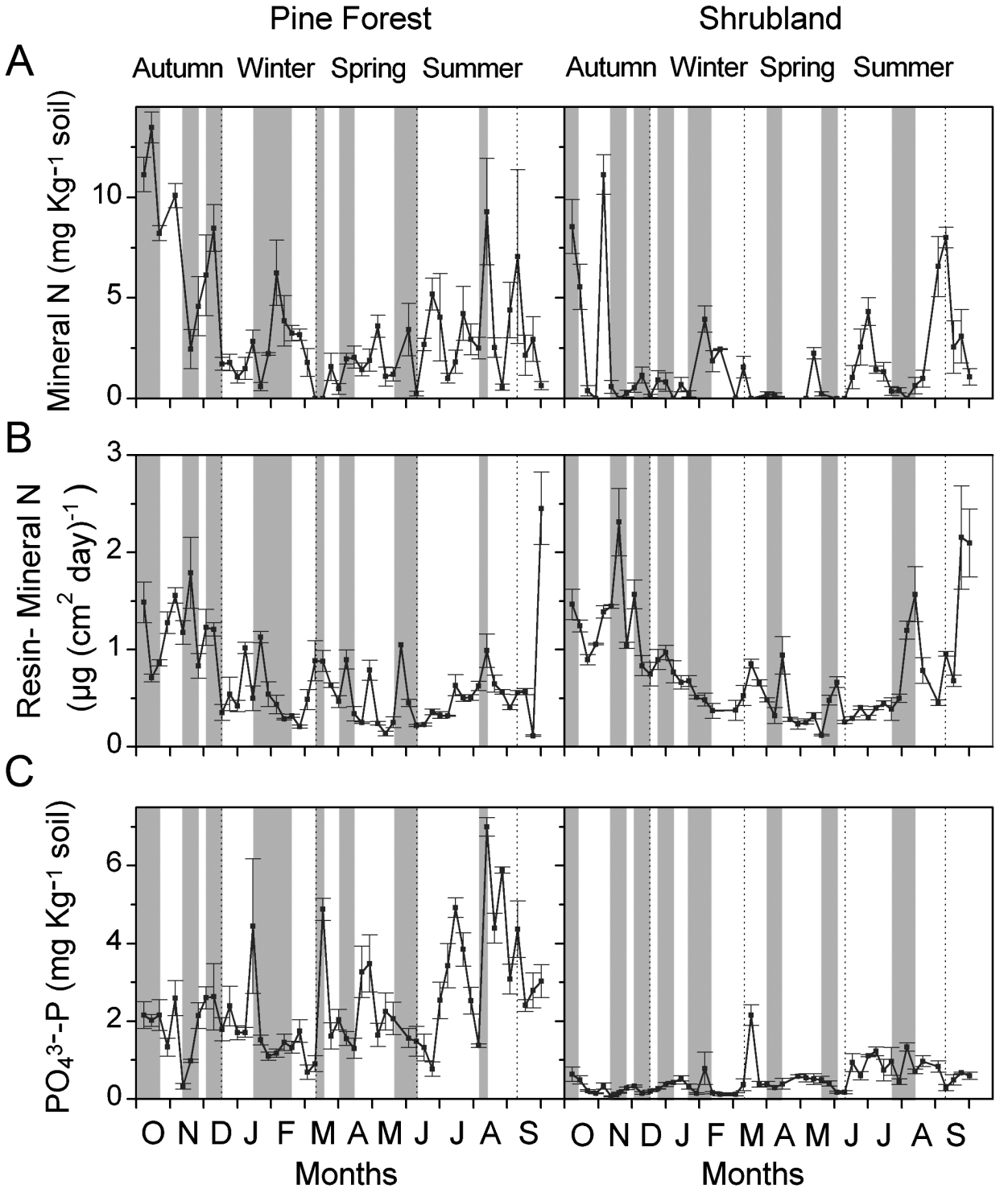
**Figure 5.** Changes of (a) mineral N, (b) resin mineral N, and (c) $PO_4^{3-}$ -P during wetting-drying cycles at each study site. Data were analysed for the entire year and separately for dry and wet soils. Error bars are ± SE, with N=8 for the

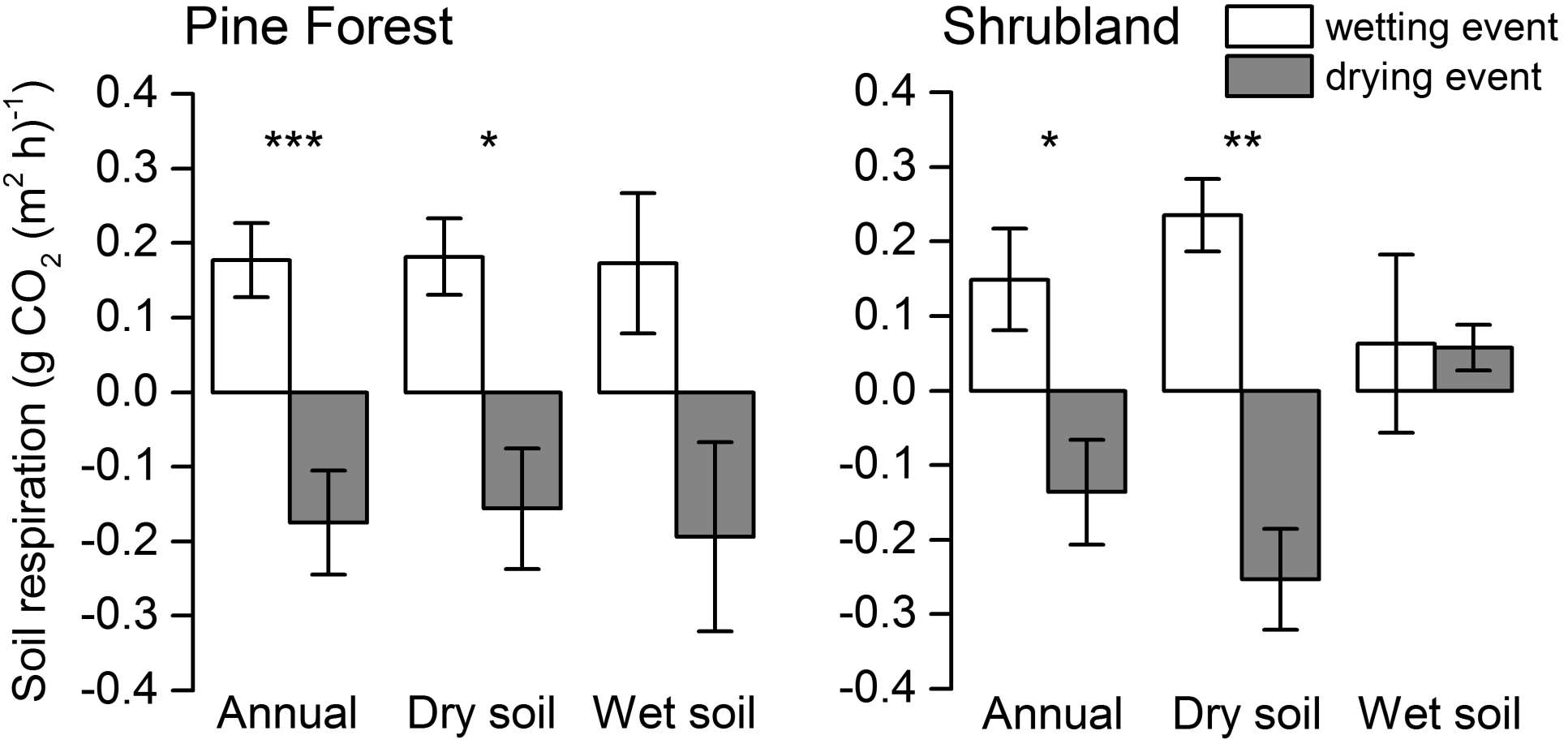
577 annual average and N=4 for dry and wet soil. Significant differences are

578 indicated as $P < 0.01$ (**).


A
Pine Forest
Shrubland
Autumn
Winter
Spring
Summer
Soil moisture in % of the WHC
100
80
60
40
20
0
B
Soil temperature (°C)
35
30
25
20
15
10
5
0
Precipitation (mm)
50
40
30
20
10
0
O N D J F M A M J J A S
Months


Pine Forest
Shrubland
Autumn
Winter
Spring
Summer
Soil respiration (g CO2(m2 h)-1)
0,0
0,2
0,4
0,6
0,8
O
N
D
J
F
M
A
M
J
J
A
S
Months


Pine Forest
Shrubland
A
Autumn Winter Spring Summer
Autumn Winter Spring Summer
Mineral N (mg Kg-1 soil)
0
5
10
B
Resin- Mineral N
(µg (cm2 day)-1 )
0
1
2
3
C
PO43-P (mg Kg-1 soil)
0
2
4
6
O N D J F M A M J J A S
O N D J F M A M J J A S
Months
Months


Pine Forest
Shrubland
wetting event
drying event
Soil respiration (g CO2 (m2 h)-1)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
***
*
*
**
Annual
Dry soil
Wet soil
Annual
Dry soil
Wet soil

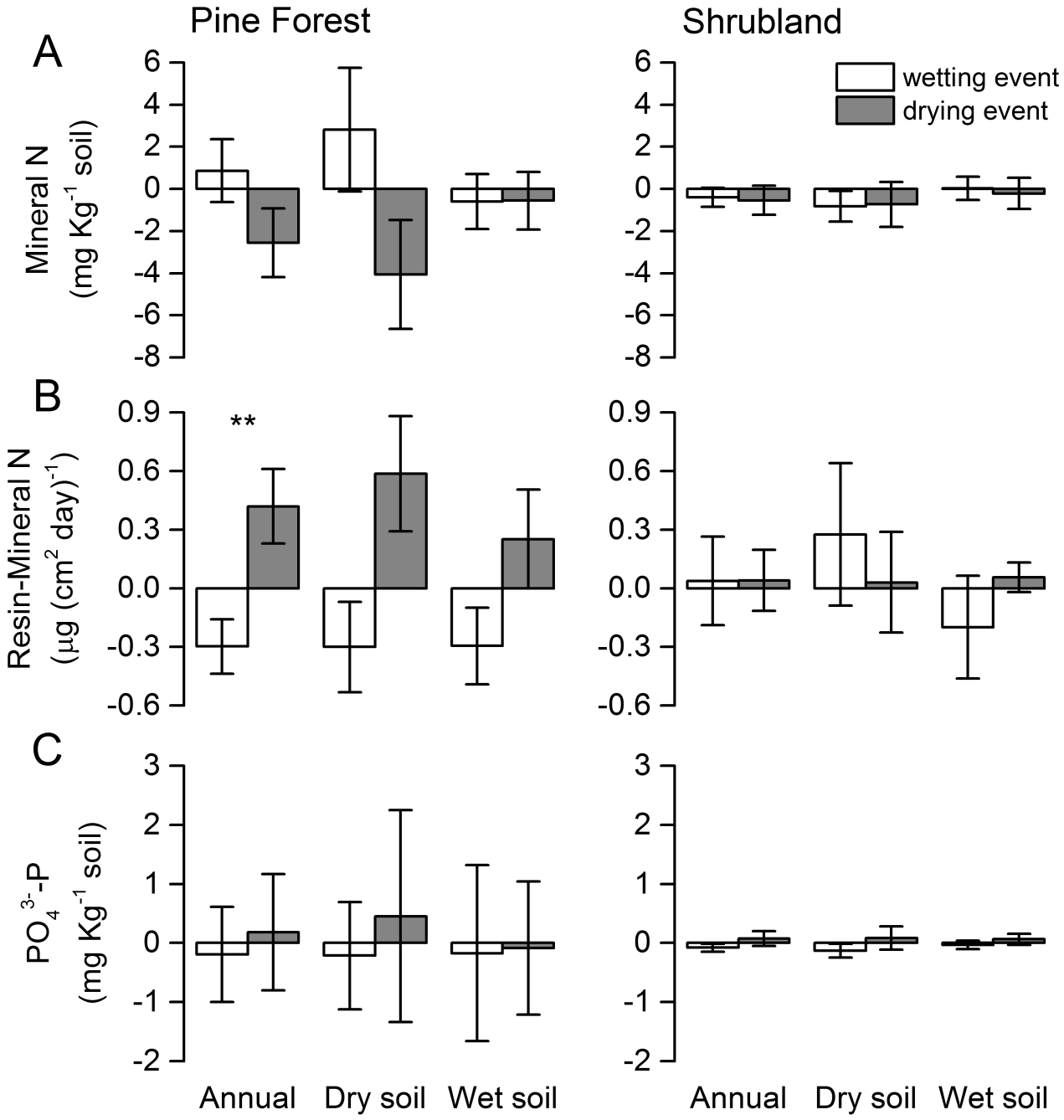

Pine Forest
Shrubland
A
B
C
wetting event
drying event
Mineral N
(mg Kg-1 soil)
Resin-Mineral N
(µg (cm2 day)-1)
PO4 3- -P
(mg Kg-1 soil)
**
Annual
Dry soil
Wet soil

**Table 1.** Soil physical and chemical properties of the top 10 cm for the pine forest and shrubland sites. Asterisks indicates significant differences between the two study sites ($p<0.05$). Variables analyzed for monitoring purpose were calculated averaging all obtained data during the whole year.

| | Pine Forest | | Shrubland | |
|---|---|---|---|---|
| Analyses for general soils description (n=24) | Mean | SE | Mean | SE |
| Clay (%)* | 23.6 | 1.71 | 6.63 | 0.80 |
| Silt (%) | 12.8 | 3.64 | 12.5 | 0.69 |
| Sand (%)* | 63.6 | 5.26 | 81.0 | 0.36 |
| Bulk density (g $cm^{-3}$)* | 1.16 | 0.07 | 1.41 | 0.09 |
| pH* | 7.2 | 0.03 | 5.49 | 0.06 |
| Organic matter (%)* | 2.84 | 0.21 | 1.91 | 0.17 |
| Phenols (mg $kg^{-1}$ soil)* | 10.42 | 1.56 | 6.61 | 0.43 |
| Hexoses (mg $kg^{-1}$ soil)* | 39.33 | .2.35 | 12.03 | 0.34 |
| Aromatic compounds (mg $kg^{-1}$ soil)* | 127.74 | 9.29 | 32.8 | 3.06 |
| Total N (%)* | 0.15 | 0.02 | 0.10 | 0.01 |
| MB-N (mg $kg^{-1}$ soil)* | 62.7 | 1.88 | 35.6 | 1.46 |
| DON (mg $kg^{-1}$ soil)* | 12.0 | 0.39 | 8.82 | 0.48 |
| Ca (meq $100g^{-1}$)* | 12.2 | 0.82 | 8.08 | 0.52 |
| Mg (meq $100g^{-1}$)* | 1.16 | 0.08 | 0.51 | 0.03 |
| K (meq $100g^{-1}$)* | 0.62 | 0.03 | 0.16 | 0.01 |
| Na (meq $100g^{-1}$)* | 0.31 | 0.01 | 0.18 | 0.02 |
| Analyses for monitoring purposes (n=312) | | | | |
| Water content (%)* | 12.4 | 0.53 | 7.98 | 0.33 |
| Water holding capacity (g $H_2O$ 100 $g^{-1}$ soil)* | 45.87 | 0.58 | 27.73 | 0.76 |
| Respiration (gr $CO_2(m^2\ h)^{-1}$) * | 0.33 | 0.01 | 0.26 | 0.01 |
| Mineral N (mg $kg^{-1}$ soil)* | 3.54 | 0.22 | 1.73 | 0.16 |
| $NH_4^+$-N (mg $kg^{-1}$ soil)* | 0.32 | 0.04 | 0.35 | 0.08 |
| $NO_3^-$-N (mg $kg^{-1}$ soil)* | 3.22 | 0.23 | 1.38 | 0.16 |

| | | | | |
|---|---|---|---|---|
| Mineral N Resins ($\mu g\ cm^{-2}\ day^{-1}$) | 0.72 | 0.03 | 0.83 | 0.04 |
| Sodium bicarbonate $PO_4^{3-}$-P ($mg\ kg^{-1}$ soil)* | 2.37 | 0.10 | 0.53 | 0.03 |
| Mineral N/ $PO_4^{3-}$-P* | 1.49 | 0.14 | 3.26 | 1.16 |

**Wetting-drying cycles influence on soil respiration in two Mediterranean ecosystems – Supplementary materials.**

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**Table S1.** Results (p values) of a linear mixed model in which the study site was treated as a fixed effect and time was treated as random effect. For simplicity, $p > 0.1$ is showed as non-significant (NS). P values below 0.05 are indicated in bold.

| | Pine Forest | | | Shrubland | | |
|---|---|---|---|---|---|---|
| | Whole year | Dry soil | Wet soil | Whole year | Dry soil | Wet soil |
| Respiration | **<0.001** | **0.029** | 0.091 | **0.014** | **0.009** | NS |
| Mineral N | NS | NS | NS | NS | NS | NS |
| Resin-Mineral N | **0.006** | 0.088 | NS | NS | NS | NS |
| $PO_4^{3-}$-P | NS | NS | NS | NS | NS | NS |

**Table S2.** Spearman correlation matrix (rho, 2-tailed) between changes in soil respiration during wetting and drying events, and the length of the wetting and drying event (LE) and the length of the prior wetting or drying event (LPE). None of the correlations were statistically significant ($p< 0.05$). N=8.

| | Pine forest | | Shrubland | |
|---|---|---|---|---|
| | Wet event | Dry event | Wet event | Dry event |
| LE | -0.3651 | -0.4568 | 0.4949 | -0.3314 |
| LPE | -0.2594 | 0.5082 | 0.3091 | -0.3849 |

**Table S3.** Pearson correlation (2-tailed) between soil respiration and soil water content (SWC) and temperature (T). Statistical differences are indicated as $p < 0.05$ (*), $p < 0.01$ (**). P values below 0.05 are indicated in bold. N=13.

| | Pine Forest | | | | Shrubland | | | |
|---|---|---|---|---|---|---|---|---|
| | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring | Summer |
| SWC | **0.862**$^{**}$ | 0.024 | **0.652**$^{*}$ | 0.276 | 0.380 | -0.308 | **0.660**$^{*}$ | **0.604**$^{*}$ |
| T | -0.258 | -0.023 | -0.600 | -0.417 | -0.053 | -0.295 | -0.441 | -0.499 |