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Soil moisture increment as a controlling variable of the “Birch effect”. Interactions with the pre-wetting soil moisture and litter addition

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Abstract

Aims

The Birch effect is a pulse in soil C and N mineralization caused by the wetting of dry soils, but the role of the soil moisture increment (ΔSWC) is still poorly understood. We quantified the relationship between ΔSWC and the Birch effect, and its interactions with pre-wetting soil moisture (*preSWC*) and substrate supply.

Methods

Two soils (clay loam and sandy loam) under a *Pinus halepensis* forest were subjected to rewetting in laboratory treatments combining different ΔSWC and *preSWC* values, with or without additional substrate (5 mg g^{-1} *P. halepensis* needles). Respiration flush (ΔR), changes in microbial biomass C (*MBC*) and net N mineralization (*NMIN*) were measured.

Results

Overall, we found a relationship with the form: $\Delta R = a\ \Delta SWC + b$, where the slope ($a$) was significant only when pre-wetting water potential was below a threshold value in the range of -

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100 to -1200 kPa. However, the threshold alone does not fully describe the role of *preSWC* in slope variability. Substrate addition modified the *ΔSWC* sensitivity of Birch effect, enhancing it in the clay loam and suppressing it in the sandy loam.

Conclusions

The intensity of the wetting is a dominant factor regulating Birch effect, and *ΔSWC* is useful for its quantification.

Keywords: Soil respiration, Nitrogen mineralization, wetting intensity, water pulses, *Pinus halepensis*, substrate limitation

Introduction

Rewetting of dry soils usually results in a pulse of C and N mineralization (Franzluebbers et al. 2000; Fierer and Schimel 2002; Austin et al. 2004; Miller et al. 2005). This effect, also known as Birch effect (Birch 1958; Jarvis et al. 2007), is attributed to the mineralization of previously unavailable, easily decomposable organic substrates (Borken and Matzner 2009). The specific origin of these substrates made available by the wetting is controversial, and thought to be due to (i) the release of intracellular compounds (osmolytes) accumulated during the dry phase as a result of microbial stress (*metabolic hypothesis* following the nomenclature of Navarro-García et al. 2012) and/or (ii) the exposure of physically protected organic matter caused by the disruption of soil aggregates (*physical hypothesis*). In most studies about drying-rewetting it is common to change soils from “dry” to “wet” conditions when examining aspects like drying-wetting frequency (Fierer and Schimel 2002; Xiang et al. 2008; Chowdhury et al. 2011b), drought length (Navarro-García et al. 2012) or temperature (Chatterjee and Jenerette 2011). Less effort has been devoted to study the wetting intensity, even though it has been recognized as an important factor that influence the mineralization pulse after a rain event (Borken and

Matzner 2009; Wu and Lee 2011; Xu and Luo 2012).

The wetting intensity is the amount of precipitation or added water per day (Borken and Matzner 2009). Theoretically, the size of the mineralization pulse is expected to rise with the amount of applied water (Borken and Matzner 2009, and references therein), as both the aggregate slacking and the release of microbial solutes should be intensified. This is in good agreement with some works where proportional relationships between wetting intensity and the mineralization response have been reported (Xu et al. 2004; Daly et al. 2009; Xu and Luo 2012). However, unclear relationships (Carbone et al. 2011), responses affected by plant cover (Sponseller 2007) or the absence of responses at low magnitude events (Cable et al. 2008), have also been reported. These contradictory results may be partly associated to the use of rainfall (or added water) under field conditions, which is the most common approach to address this question (Xu et al. 2004; Yuste et al. 2005; Sponseller 2007; Xu and Luo 2012). This approach involves uncertainty, due to some confounding factors like the interception by vegetation or runoff and leakage losses. Schmitt et al. (2010) reported little effect of rewetting size on microorganism activity due to the heterogeneous water availability caused by preferential flow paths and hydrophobicity. In this context, a more accurate approach for the quantification of the intensity of a wetting is using the increment of soil moisture caused by the event (ΔSWC, in g g^{-1}) as a variable. The use of this variable can overcome the abovementioned problems, since it focuses directly on the changes in the soil water pool. Nowadays, ΔSWC can be easily measured due to the development of technical devices that allow accurate, continuous measurement of changes in soil moisture. This enhances its potential as a predictive variable, particularly for the inclusion of Birch effect in biogeochemical models.

The increment of soil moisture is upper-bounded by saturation, and highly dependent on the pre-wetting soil moisture (*preSWC*). The higher the *preSWC*, the lower ΔSWC could possibly be. As a consequence, the interaction between these two factors makes the picture quite complex, suggesting the study of the two variables together. Earlier works had shown that a high soil water content before wetting potentially decreases the cumulative C and net N mineralization rates after the rewetting (Borken and Matzner 2009). As a soil dries and water potential becomes more negative before the wetting, both physical and metabolic processes would promote the Birch effect (Chowdhury et al. 2011a). On the one hand, the process of aggregate slacking is induced by air drying soil prior to fast rewetting (Denef et al. 2001). On the other hand, at reduced water potentials soil microorganisms acclimate by accumulating more osmolytes (Harris 1981), which are released following the wetting to avoid cell lysis (Halverson et al. 2000). All the same, a key question regarding this issue is how dry must be a soil to observe the Birch effect. Some authors suggest that an extra increase in mineralization rate after the rewetting appears only when the *preSWC* is under a threshold value (Fischer 2009; Kim et al. 2010 and references therein). Nevertheless, this issue remains poorly understood. Moreover, to our knowledge no previous studies have quantified the interactive effects of both *ΔSWC* and *preSWC* on the mineralization response to rewetting.

The size of the Birch effect also depends on the level of substrate availability (Berryman et al. 2013). The substrate input becomes a primary controlling factor in determining N and C process rates in areas with low organic matter inputs and nutrient-poor soils (McIntyre et al. 2009), where the Birch effect is particularly important (Austin et al. 2004; Jarvis et al. 2007). Previous works have shown that the amendment with leaf litter generally enhances the microbial responses to rewetting. McIntyre et al. (2009) reported that the respiration rate after a wetting in substrate-amended soils doubled that of non-amended treatments. Miller et al. (2005) observed in rewetted soils that a 10% of increase in soil C due to a previous litter addition increased the CO_2 efflux in 60%, and indicated that the amendment enhanced the initial breakdown of the indigenous soil C, suggesting a “priming effect”. Therefore, substrate quantity may also be an important factor in determining the microbial responses to *ΔSWC*, as the mineralization responses to rewetting increase with soil C content (Harrison-Kirk et al. 2013).

The aim of this study is to offer new insights about the *ΔSWC* sensitivity of the C and N mineralization pulse after a rewetting. Field-measured soil moisture data recorded for at least two years in two soils underneath Aleppo pine stands (*Pinus halepensis* Miller) were used to classify the rewetting events according to their soil moisture increment and pre-wetting soil moisture. The more frequent *preSWC* and *ΔSWC* combinations observed in the field were reflected in laboratory incubations using soils from the same sites with and without additional substrate (*P. halepensis* needles). We hypothesize that: (i) the size of the fast mineralization pulse will have a positive and proportional relationship with *ΔSWC* because this variable directly relates to the changes in the soil water availability, avoiding the hindering factors associated to rainfall; (ii) the relationship between Birch effect and *ΔSWC* will be intensified along with *preSWC* decrease, because both microorganisms and aggregates are exposed to lower water potentials (iii) the litter addition will enhance the sensitivity to *ΔSWC* due to the increase in C availability.

Material and methods

Soils description and soil moisture measurements

The two soils used for this study are located 80 km inland from Valencia, eastern Spain (39°49’ N; 1°05’ W, 980 m a.s.l.). These sites are approximately 3 km far away from each other, and were selected because they have the same vegetation community, canopy characteristics and similar climate, but contrasting soil properties. These soils will be referred to as Chelva and Tuéjar. The Chelva soil is an Albic Luvisol developed on pisolitic microcrystalline limestone, with Leptosols and Calcisols outcrop. The Tuéjar soil is a Rendzic Leptosol, developed on calcareous rock, with outcrop of Albic Luvisols and Calcaric Regosols (GVA 1995). The main characteristics of two soils are summarized in Table 1. Soils were collected from underneath an

Aleppo pine stand, with a mean density of 830 trees ha^{-1} and an average age of 60 years. The understory shrub community is dominated by *Quercus coccifera* L., and *Juniperus spp*.

The climate is Mediterranean type with hot and dry summers. Mean annual air temperature is 12.5°C and mean annual precipitation is 574 mm, which occurs mainly in autumn and spring. Soil temperature and humidity were recorded by two dataloggers (EM50, Decagon Devices, Pullman, WA, USA), one per soil type. Each datalogger is attached to a rainfall recorder used to measure throughfall (ECH2O rain, Decagon Devices, Pullman, WA, USA), a 5 cm depth soil temperature probe (RT-1, Decagon Devices, Pullman, WA, USA) and a soil moisture probe at the same depth (ECH2O 10-HS, Decagon Devices, Pullman, WA, USA). All data were measured at hourly intervals. Daily values of soil moisture were obtained as the value measured at 6:00 A.M. (Cobos and Campbell 2007). The time series include the 02/19/2009 to 02/18/2011 period for Chelva (2 years) and the 03/21/2007 to 03/20/2010 period for Tuéjar (3 years).

Pre-wetting soil moisture and soil moisture increment combinations

We used the daily time series of field measured soil moisture (data not shown) to identify the pattern of soil rewettings caused by natural rain events. Three variables were used to do so. Pre-wetting soil moisture (*preSWC*, in g g^{-1}) is the soil water content the day before a wetting event, whereas final soil moisture (*postSWC*, in g g^{-1}) is the gravimetric moisture reached after the wetting. Soil moisture increment (ΔSWC) was obtained as $\Delta SWC = postSWC - preSWC$. Soil water is a continuous variable, so we discretized it in 0.05 g g^{-1} width intervals for the classification of rewetting events. The choice of the intervals is somewhat arbitrary, but our preliminary analysis suggested this discretization would be enough in terms of capturing rewetting variability. The frequency of every *preSWC* and ΔSWC combination (hereinafter rewetting combinations) observed in the field and reproduced in the incubations, are shown in Table 2. All the rewetting combinations observed when soil temperature was below 10°C or with $postSWC >$ 60% of soil porosity (0.25 g g^{-1} for Chelva; 0.35 g g^{-1} for Tuéjar), were discarded. This was done to avoid low temperature limitations and anoxic conditions. As a consequence, there are more rewetting combinations for Tuéjar soil than for Chelva.

Experimental setup

We used a factorial design of litter addition by rewetting combination for each soil type. A composite sample of each soil was obtained by mixing six cores (0-10 cm) randomly taken from a 2x2 m^2 area after removing the O horizon in August 2011. Soils were homogenized and sieved through 4 mm. Ten grams of soil (dry weight basis) were set in 40 mL Erlenmeyer flasks. Fresh fallen brown *P. halepensis* needles were also collected in August 2011, after the period of maximum litter production (García-Plé et al. 1995). Litter samples were air dried, milled and sieved to 500 µm. The chemical characteristics of litter are shown in Table 1. The amendment treatments were added to half of the flasks, consisting of 0.05 g dry litter, being this amount equivalent to one year of the litterfall rate measured in these sites (Table 1). All these preprocessing tasks described were made the same day that the samples were taken in the field. Then, flasks were covered and soils were incubated for a three day equilibration period with the original field soil moisture (pre-incubation period).

After the three day pre-incubation period, we applied a double wetting scheme to all the flasks. The objective of the first wetting was to achieve the *preSWC* levels shown in Table 2. The first wetting consisted in bringing soils to 60% of soil porosity, namely 0.35 and 0.25 g g^{-1} for Tuéjar and Chelva, respectively. Then, flasks were uncovered to allow drying. When soils gradually reached the corresponding value of *preSWC*, the second wetting was applied. The objective of the second wetting is to reproduce the rewetting combinations defined in Table 2, so different amounts of water were added in accordance with these combinations. After that, another drying period started. The experiment finished when the last flask was completely dry, which corresponds to treatment A6. All wetting events were achieved by adding deionized water with an automatic pipette, and gravimetric soil moisture was monitored periodically by weighting the flasks. An incubation chamber (MLR-350H, Sanyo Electric Co., Oizumi-Machi, Japan) set at 25 ºC and 70 % relative humidity was used throughout the experiment. The temperature and relative humidity were chosen with the objective of replicating as close as possible the rates of soil drying observed in the field for both soils.

In this experiment, rewetting combinations were defined as a function of gravimetric water content, not water potential. This was due to the difficulty to reproduce accurately changes in water potential, due to the hysteresis associated with frequent drying-rewetting events (e.g. Fierer and Schimel 2002). However, as accessibility of water to the organisms is determined by water potential, soil water retention curves were obtained by equilibration at 0, -10, -20, -30, -100, -300 and -1500 kPa in samples of the same soils (Richards 1965). In addition, the water potential of the air dry soils were measured by the dew point method (Scanlon et al. 2002), employing a water activity meter (Aqualab series 3, Decagon Devices, Pullman, WA, USA). All the data were adjusted to the Campbell model (Campbell 1974):

$$\psi = a\,(\theta_g/\theta_{gsat})^{-b}$$

Where: ψ= matric suction (kPa); θ_g= water content (g g^{-1}); θ_{gsat}= saturation water content (g g^{-1}); a,b= equation parameters. The parameters of the model were a= -2.406, b= 4.5100, θ_{gsat}= 0.54 for Tuéjar and a'= -0.222, b'= 4.1291, θ'_{gsat}= 0.40 for Chelva.

Measurements

Respiration was measured by covering the flasks with rubber septa for 48 h. Respiration rate in that period was calculated from the increment in % CO_2 in the headspace volume of the flask, which was measured with a CO_2 sensor (Checkpoint, PBI Dansensor, Ringsted, Denmark). Soil respiration rate was measured in the pre-incubation period, and immediately after the first wetting. The respiration rate following the second wetting (R) was also measured. Additionally, the increment in respiration caused by the second rewetting (ΔR) was obtained as the difference between R and the previous value for respiration rate measured just before the rewetting. During drying periods, respiration rate was measured approximately on a weekly basis, interrupting the periods of drying (flasks uncovered, ≈5 days) with intervening periods of CO_2 accumulation (flasks covered for 48 h).

Microbial biomass C (*MBC*) was measured in rewetting combinations A1, A3, A6, B1, B3 and B4 using the chloroform fumigation-extraction technique (Vance et al. 1987, modified by Wu et al. 1990). The extraction was made 48 h after the second wetting.

To obtain net N mineralization (*NMIN*), inorganic N pools were extracted at the beginning of the experiment (the same day the soil was collected in the field) and at the end of the incubation period (128 days). Mineral N was extracted shaking each sample with 100 mL of 2M KCl. Soil extract was analyzed for $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ in a flow injection analyzer (FIAStar 5000, Foss Tecator, Höganäs, Sweden). Net N mineralization was measured in the A2, A4, A5, B2, C1, C2, C3, D1, D2, D3 and E1 rewetting combinations.

Statistical analyses

The effect of ΔSWC on ΔR was fitted by a separate linear regression for each *preSWC*. Statistical differences between the regression lines (slopes and intercepts) were performed for each combination of soil type and amendment. In addition, the R data was analyzed using one-way ANOVAs with *preSWC* as the factor for each *postSWC* level. To examine the effects of amendment more closely, a two-way analysis of variance (ANOVA) was used in each soil to determine the effects of rewetting combination x amendment on the variables R, *MBC* and *NMIN*. Statistical differences ($P<0.05$) between means were tested using least significant difference (LSD) analyses. All statistical analyses were performed with Statgraphics Plus version XVI.

Results

Soil moisture evolution during incubation

The water content of the soil samples collected in the field in Chelva soil was 0.08 g g^{-1} whereas at Tuéjar was 0.20 g g^{-1}. Overall, after the first wetting to 0.25 g g^{-1}, Chelva soil lost water gradually with an average rate of 0.003 g g^{-1} per day. In Tuéjar soil, after the first wetting (0.35 g g^{-1}), drying was slower at the beginning, taking 26 days to reach a soil moisture of 0.25 g g^{-1}. Thereafter, the drying process was accelerated, and 28 additional days were necessary to achieve soil moisture of 0.10 g g^{-1}. Soil moisture dynamics along the experiment is available online as supplementary data.

Soil respiration

The first wetting (common for all treatments) caused different responses of soil respiration in both unamended soils, with increments compared to the initial rates reaching a value of 20% in Chelva and 70% in Tuéjar. Moreover, in Chelva amended soil the first wetting caused a slight decrease in respiration (3%) compared to the rate measured before the wetting. In contrast, in Tuéjar soil the addition of litter enhanced the response to the first wetting, which caused an increase in respiration of 90%.

There were also differences in respiration dynamics during the drying periods. For Chelva soil, a steady decline in respiration rates was observed following the decrease in soil moisture, until it reached a value of 0.05 g g^{-1}. In contrast, respiration in Tuéjar soils declined more rapidly in the first drying period, reaching undetectable values at day 22, when soil moisture was 0.30 g g^{-1}. Non-zero respiration rates were only measured again in the 48 h after the second wetting, both in amended and unamended Tuéjar soils. After that 48 h flush, respiration rates were undetectable until the end of incubation, whatever the rewetting combination. The soil respiration evolution during the incubation is also available online as supplementary data.

Our results showed that both amendment and rewetting combination significantly affected the respiration after the second wetting in the two soils (Table 3). However, the main factor was different for each soil. For Chelva, the amendment explained more than 70% of variance, whereas for Tuéjar the rewetting combination explained 89%. To facilitate the identification of Birch effect, we analyzed differences in R grouping the data by *postSWC* for Chelva (Table 4) and Tuéjar (Table 5) soils. Since the soils with the same *postSWC* were incubated at the same temperature and moisture for the 48 h following the second rewetting, the significant differences in R could be attributed mainly to the extra mineralization pulse. In Chelva unamended soil, the main differences were found between the *preSWC*=0.05 g g^{-1} and the rest of pre-wetting levels (Table 4). Focusing in the *postSWC*=0.25 g g^{-1} case, only the soil exposed to the lowest *preSWC* (0.05 g g^{-1}) was significantly different from the others (P=0.0006). Hence, we identified a *preSWC* threshold value between 0.05 g g^{-1} and 0.10 g g^{-1}, equivalent to -1189 and -68 kPa in terms of water potential (Table 4). In Chelva amended soil, however, *preSWC* showed no significant effect on respiration rate (Table 4), indicating the absence of Birch effect. In Tuéjar soil, significant differences in respiration rates were found among all the *preSWC*<0.20 g g^{-1} treatments (Table 5). Thus, Birch effect was detected in unamended Tuéjar soil when *preSWC*<0.20 g g^{-1}, with the threshold in this case located between 0.15-0.20 g g^{-1} (-776 to -212 kPa, Table 5). Moreover, the highest respiration values were observed in the most water-stressed rewetting combinations (*preSWC*=0.05 g g^{-1}, Table 5), that at least doubled the rates measured in most other cases.

Overall, a statistically significant linear relationship was found between the increment in respiration rate caused by the second rewetting (ΔR) and ΔSWC (Fig. 1). Comparison of parameters between *preSWC* levels revealed significant differences between the slopes of each soil x amendment combination ($P<0.01$), except for Chelva amended soil ($P=0.4756$). In Chelva unamended soil the significance of the linear relationship is restricted to the driest pre-wetting situation (*preSWC*=0.05 g g^{-1}). Furthermore, in Tuéjar soil all the slopes were significantly different from zero except for amended soil with *preSWC*=0.20 g g^{-1} ($P=0.4634$). It should be noted that in Tuéjar unamended soil with *preSWC*=0.05 g g^{-1} we found no significant differences in *R* between the ΔSWC=0.25 and 0.30 g g^{-1} treatments. Thus, the relationship of ΔR with ΔSWC was linear only up to ΔSWC=0.25 g g^{-1} (Fig. 1c).

Microbial biomass C

The size of the microbial C pool 48 h after the second rewetting was 153.8 ± 16.6 µg C g^{-1} in Chelva soil (Fig. 2a), and 316.5 ± 38.7 µg C g^{-1} in Tuéjar soil (Fig. 2b), corresponding approximately to 1% of their respective total organic C (Table 1). In Chelva soil, the amendment was a more important factor than the rewetting combination in explaining the variability of *MBC* (Table 3). For this soil, the amendment increased *MBC* 30% on average respect to unamended. In unamended Tuéjar soil, however, there was a different response to water addition in the two *preSWC* levels tested in this experiment. For a *preSWC* level of 0.10 g g^{-1}, there was a significant increase of *MBC* according to the increase in ΔSWC. However, in the lowest pre-wetting moisture level the quantity of water added had a negligible effect on the microbial C.

N mineralization

The amendment was the factor that explained the main part of variance of the net N mineralization measured after 128 days of incubation in both soils (Table 3). The addition of litter decreased N mineralization both in Tuéjar and Chelva soils, causing net immobilization mainly in the latter (Fig. 3). Overall, the ΔSWC effects were more evident in C than in N mineralization, because the latter aggregates the effects of wetting and drying periods over the 128 days of incubation. Nevertheless, the effects of ΔSWC on *NMIN* were parallel to those on C mineralization. The influence of rewetting combination was higher in Tuéjar soil compared to Chelva (Table 3). In particular, in both Tuéjar amended and unamended soils *NMIN* responded significantly to ΔSWC only when $preSWC$=0.05 g g^{-1} (Fig. 3b).

Discussion

Soil moisture increment as a controlling variable of Birch effect

The results of this experiment show that the soil moisture increment strongly influences the magnitude of the mineralization pulse after the wetting. Particularly for C mineralization, the general pattern was a linear relationship between the increment in soil water content caused by the rewetting and the resulting respiration pulse (Fig. 1). This general result is consistent with the idea that preferential flow paths and hydrophobicity are the main interfering factors between wetting intensity and the mineralization response (Muhr et al. 2010; Schmitt et al. 2010), factors that are avoided by using ΔSWC as variable. This is further supported by the results of Daly et al. (2009), in a work where both rainfall and ΔSWC were measured. They reported linear relationships between precipitation size and respiration after the wetting, but also indicate that the precipitation and soil moisture increment were linearly related.

Despite the foregoing, the data in Tuéjar unamended soil for the lowest value of *preSWC* contradict our first hypothesis. For that particular case, we found that respiration increased as a non-linear function of soil moisture increment, reaching an asymptote at *ΔSWC*=0.25 g g^{-1} (Fig. 1c; table 5). There are some explanations to the limitation of the CO_2 pulse size for high values of *ΔSWC*. Firstly, the existence of oxygen limitation due to the high value of *postSWC* reached. Secondly, in case that the “physical hypothesis” mechanism occurs, all the aggregates could already be disrupted by swelling with an increment in soil moisture of 0.25 g g^{-1}, which implies that larger *ΔSWC*´s do not result in increases in CO_2 efflux. Thirdly, the intense and abrupt change in water potential in the *ΔSWC*=0.30 g g^{-1} treatment could have favored an increase in the death of microorganisms by cell lysis. This is expected to increase Birch effect, as more osmolytes would be released (Kieft et al. 1987). However, it is also possible that a reduction in the number of surviving microbes after the wetting could negatively affect to the community capacity to utilize the substrate immediately, limiting the mineralization pulse. As we are not able to definitely identify the source of the substrates, it is difficult to establish the true reason for the observed limitation in our experiment.

In summary, the results suggest that some interfering factors between wetting intensity and the mineralization pulse can be avoided by using *ΔSWC* as variable, but not necessarily all of them. We observed evidence of limitations that could not be ascribed to heterogeneous soil water availability. We may conclude that monitoring both rainfall and the soil moisture changes is necessary to identify the relative importance of the different factors involved in the microbial flush caused by rewetting.

Interactions between preSWC and ΔSWC on the Birch effect

As expected, changes in the pre-wetting soil moisture resulted in significant modifications in the sensitivity to *ΔSWC* in both soils. Consistent with other works, the severity of drought increased the response of soil CO_2 efflux (Cable et al. 2008; Kim et al. 2010;Unger et al. 2010, Carbone et al. 2011; Chowdhury et al. 2011a). Interestingly, Birch effect was only found when the pre-wetting water potential was below a threshold in the approximate range of -100 to -1000 kPa for the two soils (tables 4 and 5). This range includes the value reported by Fischer (2009), who found remarkable rewetting effects on respiration when water potential was below -630 kPa. However, particularly in Tuéjar soil, the different responses observed in the range of water potentials tested in this experiment suggest a greater level of complexity in the role of *preSWC*. In this soil, the stimulation response was clearly stronger when *preSWC*=0.05 g g^{-1} compared to the other *preSWC* values (Figs. 1 and 3). Similar responses were found by Rey et al. (2005), who added water to soils previously incubated at different water contents, and reported that the response to wetting in the previously driest soil was an order of magnitude higher than for the rest of pre-wetting treatments.

The microbial biomass responses to *ΔSWC* in Tuéjar unamended soil were also different depending on *preSWC* (Fig 2b). It is possible that the drying intensity before the wetting event altered the relative contribution of the physical and the metabolic hypotheses to Birch effect, as depicted in Fig. 4. When the pre-wetting soil moisture was 0.10 g g^{-1} the *MBC* significantly increased with the wetting intensity (Fig. 2b). Thus, it is not possible that the “extra” respiration observed with the increase in *ΔSWC* at this level of pre-wetting soil moisture had come from microbial stress. This supports that Birch effect was mainly due to physical disruption processes. In light of our respiration results, it seems that the aggregates would start to brake and liberate SOM after a wetting when the soil previously dries up to 0.15 g g^{-1}. This is consistent with the results reported in a soil with similar SOM by Haynes and Swift (1990) which found a rapid decrease in aggregate stability when soil dries from 18% to 10% soil water content. Conversely, when *preSWC*= 0.05 g g^{-1}(<-40000 kPa), the *MBC* was not significantly affected by the *ΔSWC* but the respiration and *NMIN* increased dramatically. As the microbial stress threshold for Mediterranean soils is approximately -10000 kPa (Manzoni et al. 2012), it is reasonable to assume that the metabolic mechanism appeared here, possibly operating simultaneously with the physical (Fig. 4). Given these lines of evidence, we propose that in unamended Tuéjar soil the mechanisms that cause Birch effect appear at different stages throughout the drying process, although this remains to be confirmed. Furthermore, in Chelva soil the source of the C pulse at rewetting cannot be definitely identified, but the influence of aggregate disruption is probably restricted to clayey soils (Borken and Matzner 2009).

Our findings indicate that care should be taken when assuming that the *preSWC* controls on Birch effect are limited to a threshold value that activates a *switch-like* mechanism, obviating the role of *preSWC* under that threshold. In-depth measurements of the impact of changes in *preSWC* under the threshold in the mineralization pulses should be made in a wider variety of soils to accumulate evidence to clarify further this aspect.

Effect of litter addition on Birch effect

Regarding litter addition, a question that arises from our work is why it prevented the Birch effect in Chelva soil (Fig. 1b; Table 4), whereas in Tuéjar soil increased the differences between slopes (Fig. 1d). We had hypothesized that litter addition would amplify the Birch effect due to the increase in C supply, but the results in Chelva soil show the opposite. In fact, in Chelva soil the litter addition caused a higher increment in organic C (with +23.6% respect to original soil organic C, Table 1) compared to Tuéjar (+9.8%). Paradoxically, perhaps the large increase in C availability itself could have been the cause that masked the respiration burst in Chelva soil. Presumably, in Chelva amended soil the excess of substrate caused by the amendment made that the extra substrates provided by drying and wetting became irrelevant to the microbial populations. As a consequence, the response to the second wetting was independent of the rewetting combination (Fig. 1b). Providing additional support to this conclusion, microbial respiration in litter is less sensitive to drying compared to mineral soil because in the former the

C supply remains active at lower water potentials (Manzoni et al. 2012).

Conversely, in Tuéjar soil the C supply by the amendment increased the sensitivity to *ΔSWC*, reinforcing the hypothesis that more C availability enhances Birch effect (Berryman et al. 2013). Moreover, in Tuéjar soil the amendment changed the *MBC* responses to *ΔSWC* (Fig. 2b), and therefore the mechanism discussed above for unamended soil depicted in Fig. 4 is not applicable to the amended samples. In particular, the absence of differences in *MBC* when *preSWC*=0.10 g g^{-1} indicate that in amended samples the “physical hypothesis” is not necessarily the main source of Birch effect. It is possible that the pre-wetting microbial stress appeared in the amended soil at higher *preSWC* values than in the unamended. The amendment could have promoted the growth of heterotrophic zymogenous soil microorganisms, and it is generally accepted that these are more susceptible to drying than the autochthonous ones (Bottner 1985; Van Gestel et al. 1993). Hence, because of a change in the microbial community composition, the amendment possibly altered the relative importance of the physical vs metabolic mechanisms in Birch effect.

Along with the stimulation of microbial activity, the amendment strongly inhibited N mineralization, as expected due to the high C:N ratio of the litter (Austin et al., 2004). The *Pinus halepensis* needles have also secondary compounds that can inhibit microbial decomposition (Fernandez et al. 2006). The N immobilization was more intense in Chelva compared to Tuéjar amended soil (Fig. 3). This is possibly related to changes in their respective microbial community compositions caused by the amendment, which can result in modifications in the microbial ability to utilize C (Butterly et al. 2009). In addition, the cumulative C fluxes integrated for the whole experiment in Chelva amended soil doubled that of Tuéjar amended (approximately 1000 vs 500 µg C-CO_2 g^{-1}; data not shown). Therefore, as more quantity of substrate with high C/N ratio was decomposed, we can expect higher immobilization rates in the former. To conclude, this experiment demonstrated that increases in substrate quantity can result both in amplifying or minimizing the relative importance of Birch effect in C and N cycling, depending on the size of the local resource pool and the seasonal availability of litterfall.

Conclusions

In this laboratory experiment we have shown that: (i) the SOM mineralization flush after a wetting increased proportionally with ΔSWC, but this relationship could be limited at high values of ΔSWC due to factors that cannot be ascribed to heterogeneous soil water availability; (ii) the ΔSWC sensitivity of the Birch effect decreased non-linearly with the pre-wetting soil moisture, and therefore it should not be simplified with a single threshold value ; and (iii) the Birch effect sensitivity to ΔSWC was also modified by the litter addition, that enhanced or minimized the importance of the mineralization pulse depending on how much C is added in comparison to the native C pool. Our results highlights that the soil microbial sensitivity to wetting intensity has a strong spatiotemporal variability, as it is soil dependent and is linked to the substrate availability. We have demonstrated that both wetting intensity and pre-wetting soil moisture can be critical factors for the C and N mineralization flush, and thus the convenience of including them in biogeochemical models. For this purpose, the ΔSWC should be used as a complementary variable, together with rainfall, for an accurate incorporation of Birch effect in C and N ecosystem balances.

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Figures

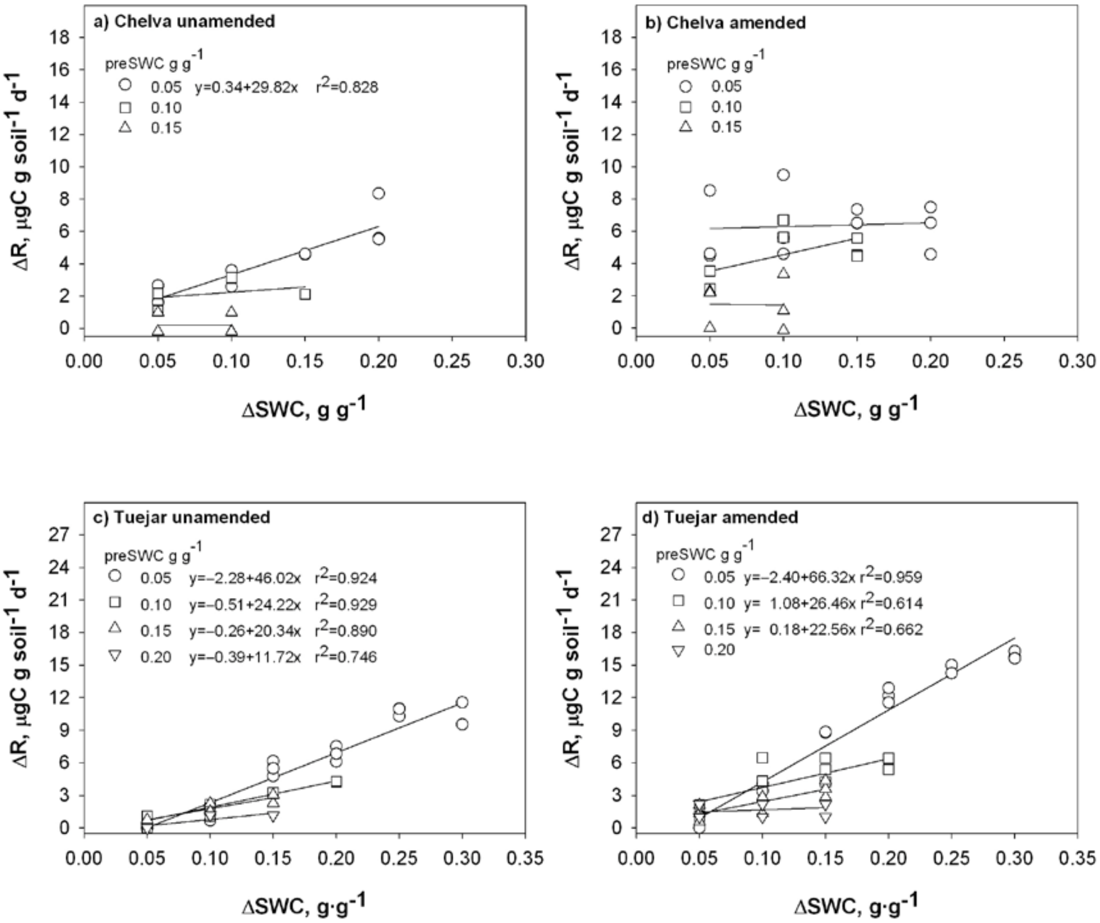


Fig 1. Relationship between the respiration increment caused by the second rewetting (*ΔR*) and soil moisture increment (*ΔSWC*) for each *preSWC* in Chelva unamended (a), Chelva amended (b), Tuéjar unamended (c) and Tuéjar amended (d) soils. Regression parameters and r^2 are shown when slope is significantly different from zero. In Tuéjar unamended (c) when *preSWC*=0.05 g g^{-1} there were no significant differences between the respiration response in *ΔSWC*=0.25 and 0.30 g g^{-1}. Therefore, *ΔR* increased non-linearly reaching an asymptote when *ΔSWC* is between 0.20 and 0.25. The linear regression analysis is showed here for comparison purposes.

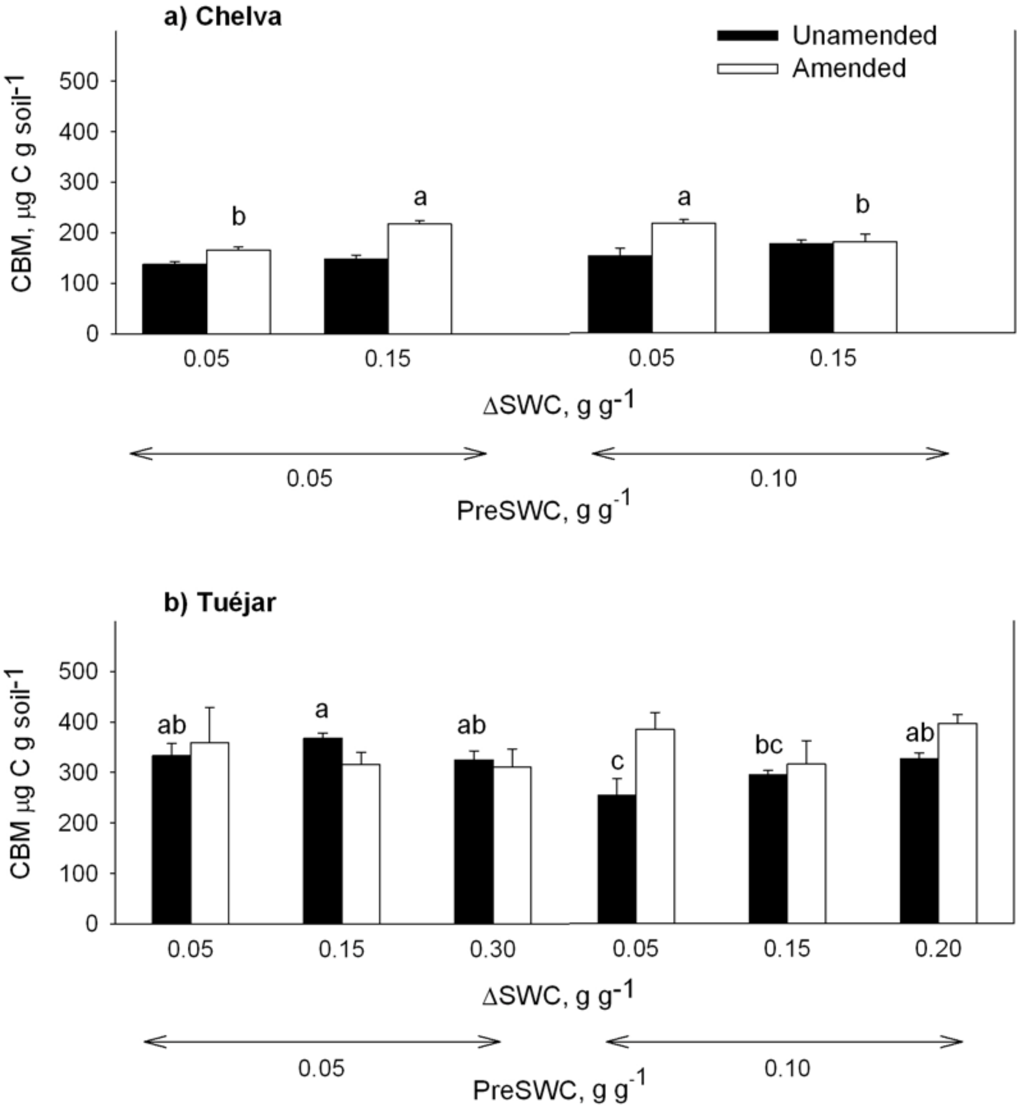


Fig. 2. Chloroform-labile C measured 48 h after the second rewetting (*MBC*) in Chelva (a) and Tuéjar (b) soils. Lower case letters denote one-way ANOVA significant differences ($P<0.05$) with rewetting combination as factor. Error bars represent SD.

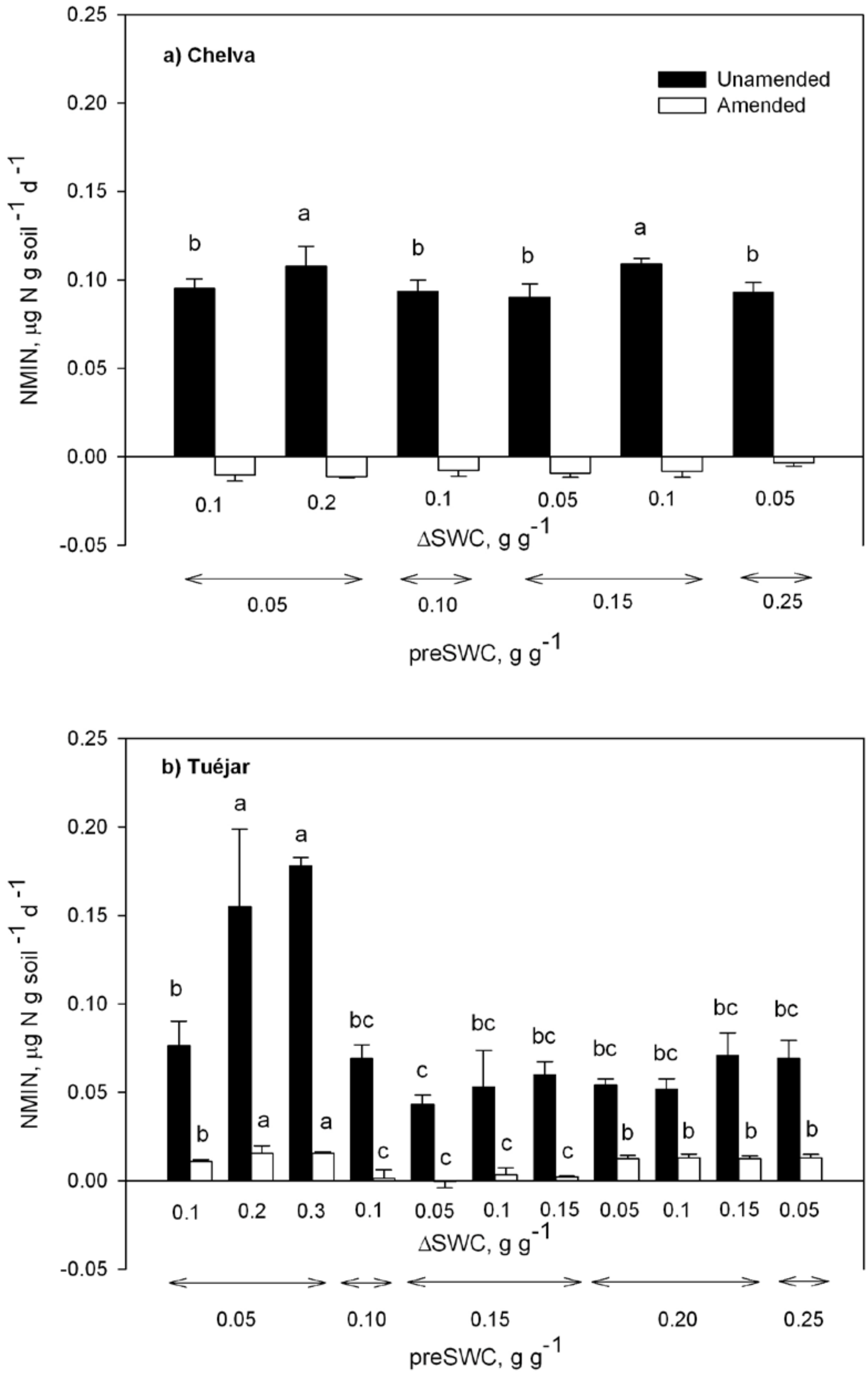

a) Chelva
Unamended
Amended
NMIN, µg N g soil -1 d -1
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
b
a
b
b
a
b
0.1
0.2
0.1
0.05
0.1
0.05
ΔSWC, g g -1
0.05
0.10
0.15
0.25
preSWC, g g -1
b) Tuéjar
NMIN, µg N g soil -1 d -1
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
b
b
a
a
a
a
bc
c
c
c
bc
c
bc
c
bc
b
bc
b
bc
b
bc
b
0.1
0.2
0.3
0.1
0.05
0.1
0.15
0.05
0.1
0.15
0.05
ΔSWC, g g -1
0.05
0.10
0.15
0.20
0.25
preSWC, g g -1

Fig. 3. Net N mineralization (*NMIN*) measured in Chelva (a) and Tuéjar (b) soils in the 128 day incubation. Lower case letters denote one-way ANOVA significant differences ($P<0.05$) with rewetting combination as factor. Error bars represent SD.

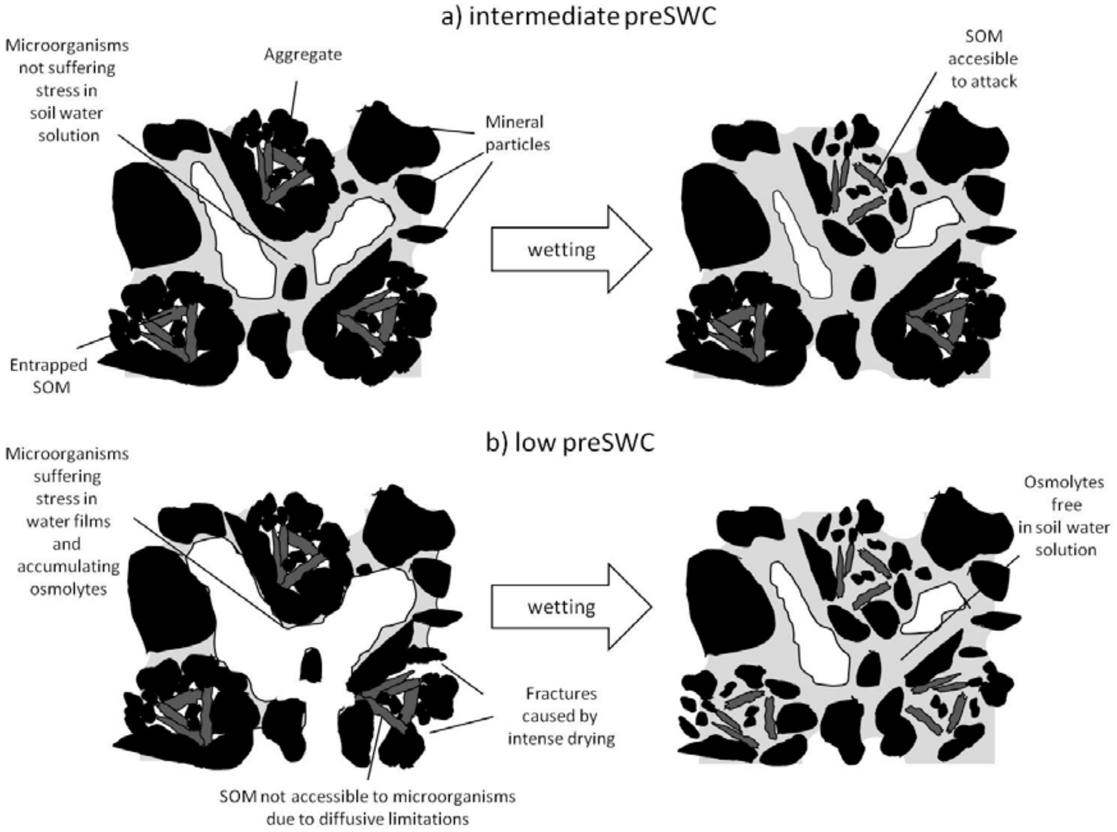
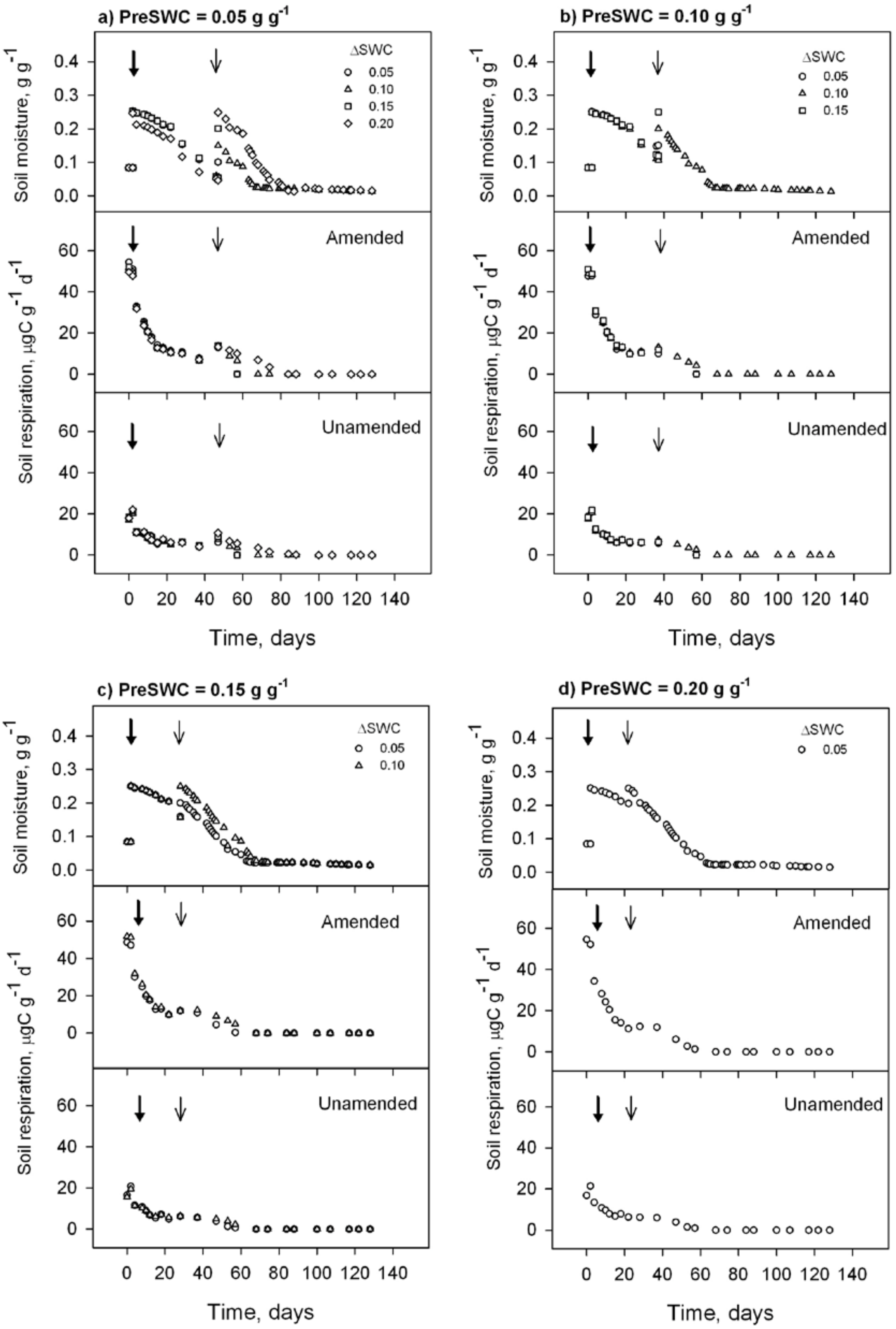
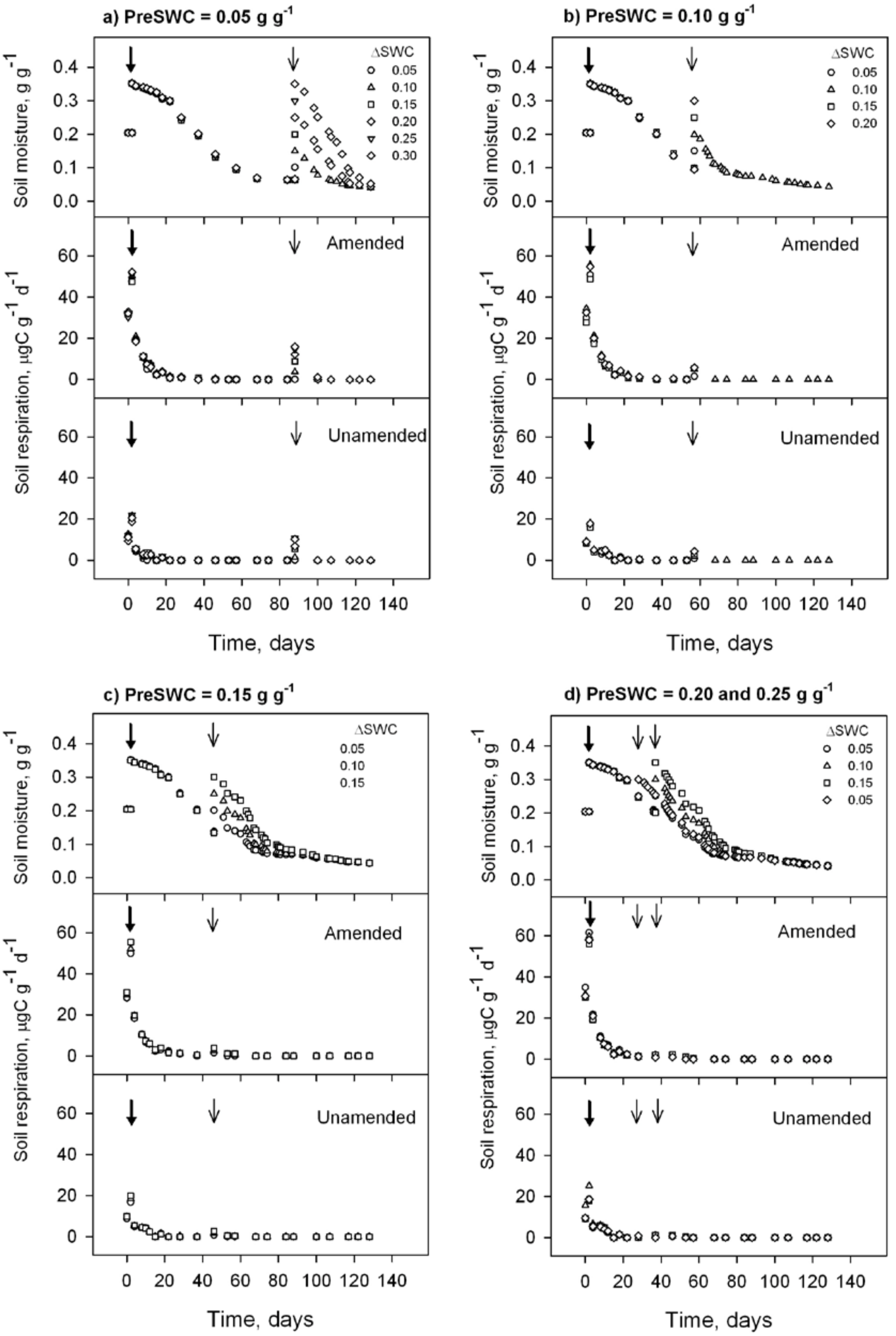


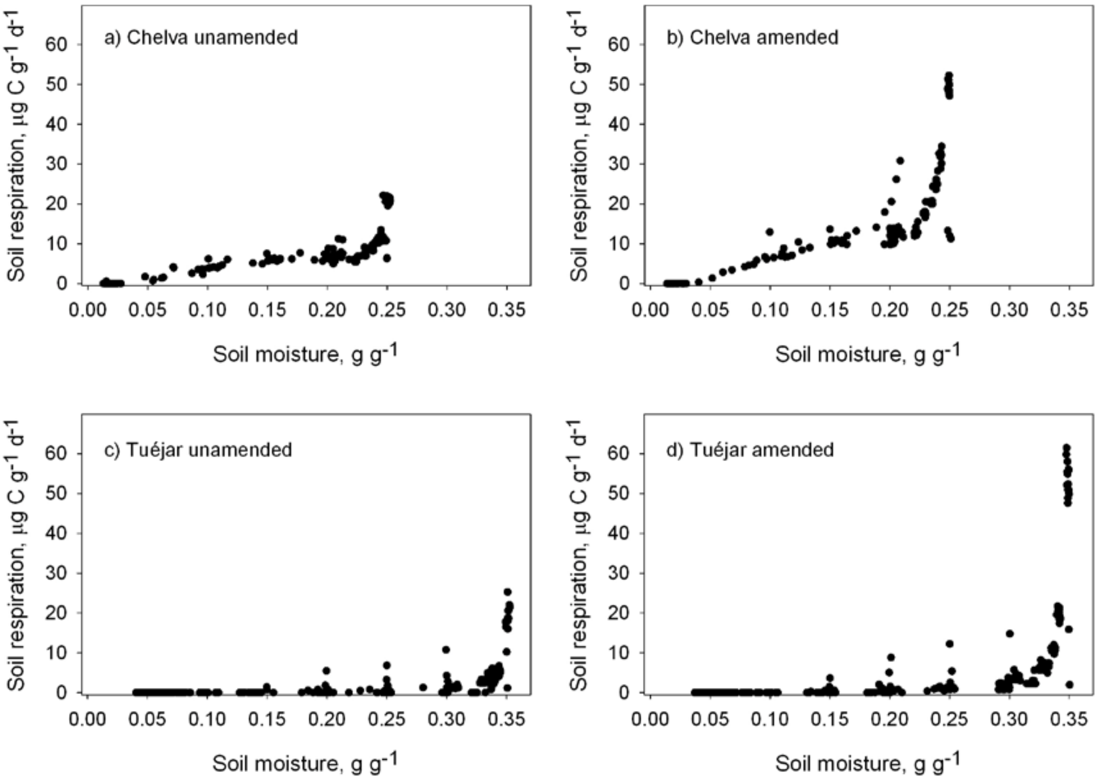
Fig. 4. Schematic conceptual model of the controlling role of *preSWC* on Birch effect suggested for incubated unamended Tuéjar soil (clay loam) in this experiment. a) At intermediate values of *preSWC* (0.10-0.15 g g^{-1}) a wetting event exposes previously unavailable SOM by physical processes, but the change in water potential does not cause a significant osmotic shock to microorganisms. b) Starting from a drier condition (*preSWC* =0.05 g g^{-1}, near air-drying) microorganisms are suffering from water stress. If a wetting is applied, “extra” cytoplasmic osmolytes are made available. Furthermore, increasing the severity of drought could enhance the accessibility to SOM after the wetting, due to (i) the aggregate destabilization during the drying process and/or the swelling and slacking of more aggregates. The wetting event is assumed with the same *ΔSWC* for (a) and (b).



Supplementary data Fig. 1. Respiration and moisture dynamics in Chelva soil for rewetting combinations with (a) *preSWC*=0.05 g g^{-1}, (b) *preSWC*=0.10 g g^{-1}, (c) *preSWC*=0.15 g g^{-1} and (d) *preSWC*=0.20 g g^{-1}. Closed arrows indicate the first wetting and open arrows the second wetting.


a) PreSWC = 0.05 g g^{-1}
b) PreSWC = 0.10 g g^{-1}
c) PreSWC = 0.15 g g^{-1}
d) PreSWC = 0.20 and 0.25 g g^{-1}
ΔSWC
0.05
0.10
0.15
0.20
0.25
0.30
Soil moisture, g g^{-1}
Soil respiration, μgC g^{-1} d^{-1}
Amended
Unamended
0.0
0.1
0.2
0.3
0.4
0
20
40
60
80
100
120
140
Time, days

Supplementary data Fig. 2. Respiration and moisture dynamics in Tuéjar soil for rewetting combinations with (a) *preSWC*=0.05 g g^{-1}, (b) *preSWC*=0.10 g g^{-1}, (c) *preSWC*=0.15 g g^{-1} and (d) *preSWC*=0.20 and 0.25 g g^{-1}. Closed arrows indicate the first wetting and open arrows the second wetting.



Supplementary data Fig.3. Relationship between respiration rate and soil moisture throughout the incubation experiment in Chelva unamended (a), Chelva amended (b), Tuéjar unamended (c) and Tuéjar amended (d) soils.

Table 1

Soil and litter characteristics for Chelva and Tuéjar sites.

	Chelva	Tuéjar
Soil chemical characteristics		
pH	8.0	8.3
Conductivity in water extract 1:5 (dS m^{-1})	0.08	0.12
Carbonates (g kg^{-1})	16	106
Organic C (g kg^{-1})	10.9	26.6
Total N (g kg^{-1})	0.77	1.26
C-to-N ratio	14.2	21.2
Soil physical characteristics		
Bulk density (g cm^{-3})	1.44	1.19
Coarse fragments (g kg^{-1})	70	181
Sand (g kg^{-1})	663	203
Silt (g kg^{-1})	157	437
Clay (g kg^{-1})	180	360
Aggregate mean weight diameter (mm)	0.46	0.99
Litter characteristics		
Litterfall rate (g m^{-2} $year^{-1}$)	305	319
Total C (g kg^{-1})	538	523
Total N (g kg^{-1})	5.54	5.70
C-to-N ratio	97.1	91.8

Table 2

Definition of rewetting combinations used in laboratory incubations and corresponding frequency observed in the field. More details in the text.

Pre-wetting soil moisture (*preSWC*±0.025 g g^{-1})	**Soil moisture increment (Δ*SWC*±0.025 g g^{-1})**	**Final soil moisture (*post-SWC*±0.025 g g^{-1})**	**Rewetting combination**[a]	**Average number of field observations per year when T>10°C** [b]	
				Tuéjar	**Chelva**
0.05	0.05	0.10	A1	1.0	1.5
	0.10	0.15	A2	0.3	0.5
	0.15	0.20	A3	0.3	1.5
	0.20	0.25	A4	0.3	1.0
	0.25	0.30	A5*	0.3	1.0
	0.30	0.35	A6*	0.0	0.0
0.10	0.05	0.15	B1	1.3	1.0
	0.10	0.20	B2	1.3	0.0
	0.15	0.25	B3	0.0	0.0
	0.20	0.30	B4*	0.3	1.0
0.15	0.05	0.20	C1	0.7	1.5
	0.10	0.25	C2	0.3	2.0
	0.15	0.30	C3*	1.0	0.0
0.20	0.05	0.25	D1	3.0	1.5
	0.10	0.30	D2*	0.3	0.0
	0.15	0.35	D3*	0.3	0.0
0.25	0.05	0.30	E1*	3.0	4.5

[a] Combinations marked with an asterisk were not applied to Chelva soil in laboratory incubations.

[b] Period of observation for Tuéjar soil: 03/21/2007-03/20/2010 (3 years). Period of observation for Chelva soil: 02/19/2009-02/18/2011 (2 years)

Table 3

Significant (P<0.05) percentages of variance explained by the factors rewetting combination, litter addition and the interaction between them, for the respiration rate after the second rewetting (*R*), the chloroform-labile C measured 48 h after the second rewetting (*MBC*) and the net N mineralization (N-NO_3^-+N-NH_4^+) measured in the 128 day incubation (*NMIN*).

Factors	**Explained variance by the factors (%)**					
	R		***MBC***		***NMIN***	
	Chelva	**Tuéjar**	**Chelva**	**Tuéjar**	**Chelva**	**Tuéjar**
Rewetting combination	15.07	89.04	21.54		0.45	23.18
Amendment	72.19	5.62	51.58	11.40	98.07	55.52
Rewetting combination x amendment		3.96	21.53	43.06	0.77	15.24

Table 4

Respiration rates after the second rewetting (μg C g^{-1} d^{-1}) sorted by *postSWC* under different *preSWC* in Chelva soil. Soil matric potentials (ψ) were estimated using the Campbell model (Campbell, 1974). Lower case letters denote one-way ANOVA significant differences (P<0.05) with *preSWC* as factor.

		Respiration rate (μg C g^{-1} d^{-1})							
		Unamended soil				**Amended soil**			
preSWC	**ψ**	***postSWC***				***postSWC***			
g g^{-1}	**kPa**	**0.25**	**0.20**	**0.15**	**0.10**	**0.25**	**0.20**	**0.15**	**0.10**
0.05	-1189.3	10.70a	8.82a	7.48a	6.16	13.24	13.83	13.60	12.91
0.10	-68.0	6.34b	7.36b	5.69b		11.90	13.03	9.86	
0.15	-12.7	6.23b	6.23c			11.94	12.00		
0.20	-3.9	6.38b				11.26			
P-value		**0.0006**	**0.0006**	**0.0221**		0.3971	0.1052	0.0740	

P<0.05 are indicated in bold.

Table 5

Respiration rates after the second rewetting (µg C g^{-1} d^{-1}) sorted by *postSWC* under different *preSWC* in Tuéjar soil. Soil matric potentials (ψ) were estimated using the Campbell model (Campbell, 1974). Lower case letters denote one-way ANOVA significant differences ($P<0.05$) with *preSWC* as factor.

preSWC	***Ψ***	**Respiration rate (µg C g^{-1} d^{-1})**											
		Unamended soil						**Amended soil**					
		postSWC						***postSWC***					
g g^{-1}	**kPa**	**0.35**	**0.30**	**0.25**	**0.20**	**0.15**	**0.10**	**0.35**	**0.30**	**0.25**	**0.20**	**0.15**	**0.10**
0.05	< -40000	10.22a	10.74a	6.83a	5.47a	1.38	0.00	15.87a	14.74a	12.20a	8.82a	3.63a	0.00
0.10	-4834.9		4.28b	3.25b	1.81b	0.72			5.73b	5.37b	5.03b	1.43b	
0.15	-776.6		2.80c	1.77c	0.76b				3.78c	2.52c	1.53c		
0.20	-212.2	1.17b	1.18d	0.00d				1.96b	1.94d	1.56c			
0.25	-77.6		0.87d						1.29d				
P-value		**0.0002**	**<0.0001**	**<0.0001**	**0.0001**	0.2889		**<0.0001**	**<0.0001**	**<0.0001**	**0.0001**	**0.0076**	

P<0.05 are indicated in bold.