The influence of leaf litter and plants on soil respiration before, during and after a severe drought

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Declaration

This thesis is my own work and it has not been submitted in substantially the same form to any other institution for the award of a higher degree.

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Abstract

Drought frequency is projected to increase under future climate change. Large-scale droughts may have a huge effect on ecosystem carbon storage but despite the importance of drought events, their effect on soil carbon dynamics are less well understood. The above-belowground linkages that affect soil dynamics in forested areas during drought and immediately after rewetting can have a substantial impact on how soil carbon is stored. Soil respiration increases with addition of organic material, which also facilitates the release of stored carbon in the soil; known as a 'priming effect'. In addition, soils that have undergone drought produce a large pulse of CO_2 when rewet; a phenomenon known as the 'Birch effect'.

I conducted lab incubations and a greenhouse experiment with poplar saplings to quantify soil carbon release during and after drought. I measured soil respiration to investigate potential interactions between the Birch and the priming effects and to establish whether post-drought soil CO_2 release is intensified or mitigated with the addition of different amounts of leaf litter and the presence or absence of tree roots.

In both experiments, soil respiration increased with litter inputs and decreased strongly during drought. However, I observed a larger pulse of soil CO_2 efflux in response to litter inputs compared to rewetting after drought. In the incubation experiments, the low carbon content of the soil explains the overriding effect of the litter treatments, because the litter inputs represented the main source of carbon and nutrients to soil microbes. In the greenhouse experiment, I observed a substantial increase in soil carbon and microbial biomass upon rewetting after

drought but no clear peak in soil CO_2 efflux. The apparent lack of a Birch effect in the greenhouse experiment is intriguing, because it suggests that plants have a mitigating effect on soil microbial responses to drought and rewetting.

Introduction

Soils are huge repositories of carbon. Terrestrial carbon stocks contain three times the amount of carbon in the atmosphere and are vulnerable to change due to human activity (Batjes 1996). The vast majority of terrestrial carbon is stored belowground in soils and yet potential changes in soil carbon pools in climate change models are often inaccurate because we lack a detailed understanding of the processes involved in soil carbon storage and release (Wieder *et al.* 2013).

Most organic carbon is introduced to the soil matrix during microbial decomposition of plant input; it is also released from the soil during this process by heterotrophic respiration. Organic carbon entering the soil is predominately part of long complex polymer chains; cellulose, proteins, hemicellulose and lignin are the most abundant forms of carbon in decaying organic matter (Swift 2001). As most soil microorganisms are heterotrophic, they are primarily limited by carbon and these large organic polymers need to be depolymerised by specialised bacteria and fungi to release the simple compounds and sugars that can be used for a source of energy for the rest of the soil microbial community (Killham 1995). After processing by decomposer organisms, most of the carbon from fresh organic matter is either: 1) incorporated into the organisms feeding off the organic matter; 2) released as CO_2 through microbial respiration; or 3) becomes part of the carbon pool in the soil (Killham 1995). The storage of carbon in soils is of critical importance because much of it is eventually stabilised, with turnover times in the range of hundreds or thousands of years (Swift 2001). Hence, the response of soils to climate change can influence ecosystem or even global carbon dynamics, and yet we still know little

about the processes underlying the storage or release of carbon from soils under different conditions.

The functional rates of the biological processes that govern soil carbon dynamics are highly dependent on environmental conditions, in particular temperature and water availability (Raich & Schlesinger 2002). Drought can result in decreased microbial biomass and activity, however this is not uniform (van Gestel *et al.* 1993; Bapiri *et al.* 2010) as some slower-growing microorganisms, such as the slower growing fungi, are thought to be more resistant to desiccation (Schimel *et al.* 2007; Bapiri *et al.* 2010). Temperature is crucial for regulating microbial activity but it also plays a part in determining the size and diversity of the microbial community: an increase in temperature alone can result in greater microbial biomass but it can also decrease the diversity of the microbial community because it favours fast-growing competitive organisms (Scheik *et al.* 2011). However, a rise in temperature combined with low precipitation can cause more severe drought in soil, leading to an overall greater loss in microbial biomass (Scheik *et al.* 2011).

Two phenomena are of particular interest for the study of soil carbon dynamics because they can release large pulses of CO_2 from soils: 1) the 'Birch effect', which occurs after rewetting of dried soils, and 2) the 'priming effect', which occurs in response to increased inputs of fresh organic matter. Both phenomena involve complex microbial, biochemical and organo-mineral interactions that have been studied for decades, but the mechanisms underlying the pulsed release of CO_2 from soils have yet to be conclusively identified.

The Birch effect was first described in experiments by H.F. Birch in the 1950s; he discovered that repeated drying and rewetting cycles in soils stimulate the mineralisation of soil organic matter, releasing carbon and mineral nitrogen (see Jarvis *et al.* 2007 for a review). Three principal mechanisms have been proposed for the pulse of CO_2 associated with the Birch effect (Jarvis *et al.* 2007 and Kim *et al.* 2012):

i) Labile organic substrate accumulates during drought, which facilitates rapid growth and expansion of the microbial community when the drought is alleviated. This substrate is derived from numerous sources such as dead roots, bacterial and fungal cells that did not survive the drought, and litter that has started to decompose on the surface of the soil. The rewetting of the soil therefore produces a pulse of labile substrate that surviving microbes can exploit, leading to rapid initial growth and activity, which in turn produces a large and sudden increase in microbial respiration. (Bottner *et al.* 1985);

ii) Intracellular osmolytes are synthesized by soil microbes to counteract the decrease in water potential as drought increases. Upon rewetting, the osmolytes are rapidly released and used as substrate because they are labile organic compounds. (Fierer andSchimel 2002)

iii) Physical mechanisms are responsible for the spike in CO_2, where drought breaks up soil aggregates, releasing physically protected organic matter accounting for part of the observed pulse in soil respiration. (Denef *et al*. 2001)

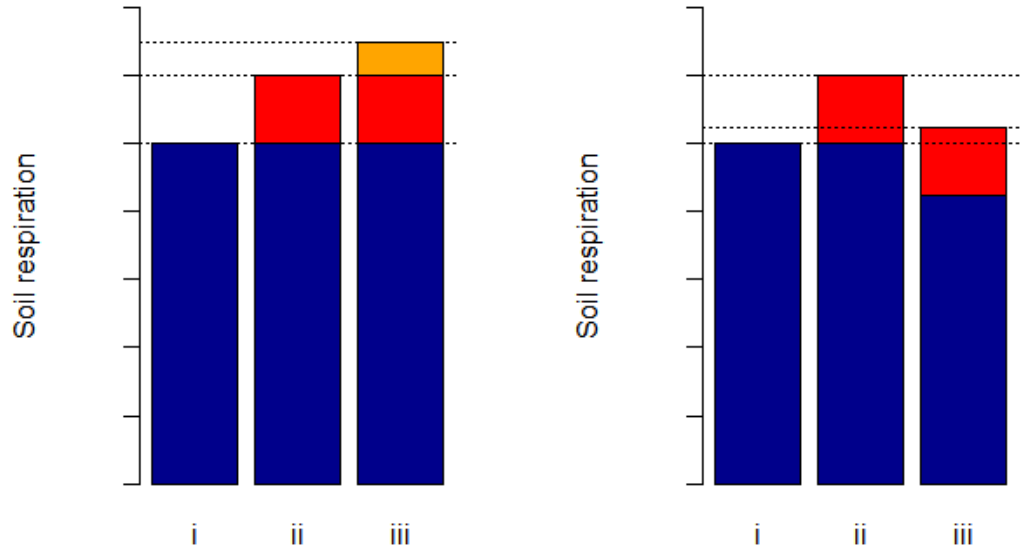
Although it is generally accepted that these mechanisms contribute to increased CO_2 efflux during Birch effects, we still lack a good understanding of the processes

involved and their wider impact on ecosystem carbon dynamics (Xiang *et al.* 2008; Kim *et al.* 2012).

The priming effect is characterised by additional decomposition of the soil organic carbon after increased inputs of easily-degradable organic material. The pulse of CO_2 observed after substrate addition is largely derived from older soil organic matter, rather than the fresh organic inputs (Kuzyakov *et al.* 2000). There are currently three widely proposed theories for the mechanisms underlying priming effects:

i) Soil microbes are typically C-limited, so the addition of easily degradable fresh organic matter provides energy and stimulates extracellular enzyme production, which results in the break down of soil organic matter (Kuzyakov *et al.* 2000).
ii) Specific microbial functional groups control the turnover of distinct pools of organic matter. Additions of more complex substrates give a competitive advantage to slow-growing groups of microorganisms, which are capable of degrading more recalcitrant soil organic matter (Fontaine *et al.* 2003).
iii) Microbial decomposition processes occur within a narrow range of the carbon-to-nitrogen ratio (C:N). Soil organic matter is mineralized to acquire nitrogen to enable the decomposition of a high-C substrate (Craine *et al.* 2007; Blagodatskaya & Kuzyakov 2008).

Studies of both "Birch" and priming effects have usually tested these theories in isolation and found evidence to support each of them. It is therefore unclear whether any single mechanism can explain each of these two effects or whether specific mechanisms apply under different conditions, soil types or ecosystems.



a) b)

Figure 1. Illustration of expected changes in soil respiration with a) positive priming and b) negative priming. i) represents CO_2 respired from the soil without additional organic input; ii) shows the soil respiration in blue plus the CO_2 released from the additional substrate in red; iii) shows changes in CO_2 measured during positive and negative priming effects, where additional CO_2 released by positive priming effects is shown in yellow; dashed lines indicate the difference in expected soil respiration with and without priming effects.

Linking Birch effects and priming effects

The mechanisms underlying Birch effects and priming effects have largely been studied in laboratory experiments. However, many of these studies were carried out without the presence of plants, even though there is strong evidence that plant roots can modify the response of soil microbes to drought (Fuchslueger *et al.* 2014) and play a key role in priming effects (Subke *et al.* 2004; Crow *et al.* 2009). Recent work demonstrates that either the presence of roots or litter alone can release carbon as CO_2 through priming effects but interactions between plant roots and litter inputs

can reduce CO_2 release and increase soil C storage (Xu *et al.* unpublished data). Given that drought also affects the growth and production of organic matter by plants as well as microbial activity, it is reasonable to assume that the presence of plants can also modify Birch effects after a drought.

There have been a number of drought experiments in grasslands, where the small size of individual plants and low vegetation allow observations of ecosystem processes. In contrast, studies of environmental change involving trees or wooded ecosystems are logistically challenging (Sayer 2014). Experiments to determine drought effects in forest soils often use partial throughfall exclusion to reduce water availability (e.g. Ogaya & Peñuelas 2007; Brando *et al.* 2008), or rainfall shelters in the understorey, which affect the surface soil and ground flora but not canopy tree growth (e.g. Borken *et al.* 2006; Cleveland *et al.* 2011). Initially, trees respond to water shortage by shifting biomass allocation from aboveground growth to root production to increase water uptake (Ryan 2011). A severe forest drought can also result in large quantities of dead plant material through leaf abscission to reduce transpiration losses and mortality both above- and belowground (Munné-Bosch & Alegre 2004). At the end of a strong drought, it is therefore conceivable that the first rainfall could cause the simultaneous occurrence of Birch effects and priming effects, as the soil is rewetted and the accumulated plant material starts to decompose. If this is the case, and priming effects and Birch effects occur simultaneously (or in close succession) at the end of a drought, the release of CO_2 could be larger than currently estimated by experiments studying either effect in isolation.

To address this, I established laboratory incubations combining different levels of leaf litter inputs with drought treatments, and a greenhouse experiment using pots with and without poplar saplings to investigate the influence of live roots on the amount of CO_2 released during Birch- and priming effects. I measured soil CO_2 efflux to assess changes in microbial activity in response to litter inputs, drought treatments and rewetting. The main aim of my experiments was to test the following hypotheses:

1) Birch effects and priming effects are additive, resulting in a larger pulse of CO_2 upon rewetting when litter inputs are increased;

2) The presence of plant roots moderates the release of CO_2 through Birch effects after severe drought;

3) Plant roots also reduce the amount of CO_2 released through priming effects in response to increased litter inputs.

Methods

Incubation experiments

Sample preparation

Fresh commercial topsoil was homogenised by sieving (2-mm mesh). Three subsamples were weighed and then dried to constant weight for 48 hours at 105°C to measure soil water content. Soil water holding capacity was determined by placing 200 g of dried soil in a 500 ml container with small holes to allow water to drain

freely. The soil was then submerged in water for 24 hours, left to drain for a further 24 hours and then weighed to determine the water holding (field) capacity.

A mixture of ash and sycamore leaf litter was collected during October 2013 from litter traps in Wytham Woods, mixed deciduous woodland in Oxfordshire UK. The litter was oven-dried to constant weight at 60ºC according to existing lab protocols, shredded, homogenised, and then sieved to retain fragments of 2-4mm diameter.

Leachate was prepared following Schreeg *et al.* (2013) with modifications. Briefly, 1.5 g of litter were shaken with 45 ml of deionised water and centrifuged at 3500 rpm for 5 mins; 90 mL of leachate were prepared each week and stored at 5 ºC until needed.

The dissolved organic content for the leachate was determined to be 240 $\mu g\ kg^{-1}$.

Incubations

For the incubations, soil samples (80 g dry weight equivalent) were placed into 56 jars (500 ml) and rewetted to 60% water holding capacity (WHC). The jars were incubated at room temperature. The controls were maintained at 60 $\pm$5% WHC throughout the experiment by measuring the weight lost by evaporation every 3-4 days and adding a corresponding amount of deionised water (dH_2O).

The soil CO_2 efflux in each incubation jar was measured at least once a week and daily after applying treatments until the CO_2 efflux settled. This was done using an infrared gas analyser with a multiplexer (Li-8100, LiCor Biosciences, Nebraska, USA) adapted for incubation vessels. Each incubation jar was capped before the measurements and then flushed for 30 seconds before measuring CO_2 efflux for 2 minutes.

Once the soil CO_2 efflux of the incubations had stabilised (after 1 week), the incubation jars were grouped into seven blocks by mean soil CO_2 efflux and two jars within each block were assigned to one of the following treatments: single-litter, double-litter, litter leachate, and controls without inputs. For the single-litter treatment, 1.22 g of chopped litter was spread evenly across the surface of the soil; the litter mass was based on field data from Wytham Woods and represents the monthly total during peak litterfall in October. Accordingly, 2.44 g of litter were added to the double-litter treatment. The leachate treatment represented the amount of leachate from rainfall and prepared litter leachate was added to these incubations to maintain WHC instead of dH_2O. To maintain 60% soil WHC while accounting for the amount of water absorbed by the litter, the target weight of the single-litter treatments was increased by 2.5 g and the double-litter treatments by 5 g.

Litter treatments were applied after 1 week and soil CO_2 flux from the incubation jars was measured every 2 days for the following week. Once soil CO_2 efflux had stabilised, half the incubations of each treatment were subjected to severe drought, whereas the other half was maintained at 60% WHC. This resulted in seven replicate incubations for each of eight treatments in a factorial design (four litter treatments $\times$ two drought treatments).

When the soil in the drought treatments reached 5% WHC, the soils were rewet to 60% WHC using dH_2O for the control, single-litter and double-litter treatments, and leachate solution for the leachate treatment. Soil CO_2 efflux of all incubations was measured daily for another seven days after rewetting.

Three to four jars per treatment were destructively sampled for soil analyses immediately before rewetting and the remaining jars were sampled at the end of the experiment, *c*. 1 week after rewetting. The litter was removed from the soil surface and the soil was homogenised by sieving prior to chemical analyses. Additional soil samples were taken from each incubation jar to determine total carbon and nitrogen content, gravimetric soil water content, and soil pH.

Litter mass was determined using 17 extra single-litter and double-litter incubation jars, which were dried to constant weight at 60 ºC before the litter was carefully separated from the soil and weighed.

Greenhouse experiment

Experimental set-up

The greenhouse experiment comprised 96 cylindrical pots (21.2 L) filled with 8.5 kg commercial topsoil (Table 1). 72 pots were planted with established poplar saplings grown from cuttings (height *c.* 30-40 cm) and 24 pots were left unplanted. All the pots were watered to field capacity. To enable measurements of soil CO_2 efflux from the pots, a soil collar made of PVC pipe (9-cm long x 10-cm diameter) was sunk into the soil to 5-cm depth in each pot; the collar was placed on the north-facing side of each pot. The pots were kept well-watered for two weeks (establishment period) to allow the saplings to recover from transplantation before the start of the experiment.

Senesced maize leaves (C content: 43.10%; N content: 1.47%) were used as a standard litter. The leaves were gathered from Raines Hall Farm in Cumbria, England after the maize harvest in October 2014. The leaves were dried to constant weight at 60ºC, shredded, homogenised, and then sieved to retain fragments of 2-4 mm diameter.

Table 1. Initial chemical and physical properties of the commercial topsoil used in the greenhouse experiment, as given by the supplier; the pH value is given for a 1:1 ratio of soil to water.

Soil chemistry		Soil texture	
Total organic matter (%)	5	Clay	23%
Available P (μg g^{-1})	4.5	Silt	35%
Available K (μg g^{-1})	135	Sand	41%
Soil pH	7.1	Fine gravel	1%

Experimental treatments and data collection

Baseline measurements of soil CO_2 efflux were taken during the initial establishment period, after which the pots were randomly subdivided into three groups and the following litter treatments were applied to 32 pots each: 'single-litter' with 19 g chopped maize, 'double-litter' with 38 g chopped maize and 'no-litter' controls without litter inputs. Soil CO_2 efflux was measured during 2 weeks after the litter treatments were applied until it stabilised. Then half the pots per litter treatment were subjected to a drought treatment and the other half were watered daily. Hence, there were six treatments in a factorial design (three litter levels and two drought levels) with a total of 12 replicate planted pots and four replicate unplanted pots per treatment.

The drought treatment was applied for three weeks, at which point the trees would not have survived any longer without water (water content: 4.85%) and all pots were watered to field capacity.

Soil CO_2 efflux, soil moisture and soil temperature measurements were taken weekly throughout the experiment using an Infrared gas analyser (Li-8100, LiCor Biosciences, Lincoln NE, USA) with a 10-cm diameter soil chamber. Soil CO_2 efflux was measured daily for four days after the start of the litter treatments and for six days after the soils were rewetted. The litter in the soil collars was carefully removed by hand before each measurement and then replaced immediately afterwards. Each soil CO_2 efflux measurement lasted 2 minutes and the system was flushed for 30 seconds between each measurement. Soil temperature readings were taken at the same time as CO_2 measurements using a 20 cm probe. Measurements of soil water content were taken at 0-6 cm using a Theta-probe (Delta-T Devices Ltd., Cambridge UK). During the drought period, additional measurements were taken at 10-cm and 20-cm depth by inserting the probe through holes drilled into the side of a subset (32) of the pots.

Soil cores (0-10 cm depth) were taken at the peak of the drought (planted pots only), and 1 week after rewetting (all pots). Soil subsamples were used to determine total carbon and nitrogen content, gravimetric soil water content, and soil pH.

Soil analyses

Soil microbial biomass was determined by chloroform fumigation following Vance *et al.* (1987) with modifications as described by Jones & Willett (2006). Briefly, two soil subsamples of 8 g (dry weight equivalent) were taken from each incubation jar. One subsample was fumigated with chloroform for 24 hours and both subsamples were extracted in 40 ml 0.5M K_2SO_4. The extracts were shaken at 200 rpm for 1 hour, centrifuged at 3000 rpm and then filtered (2.5 µm). The extracts were stored at 5 ºC until analysis for total carbon.

For total soil carbon and nitrogen content, subsamples were dried at 60 ºC for 24 hours and ground using a ball mill before analysis on a Vario ELIII Element Analyser (Elementar, Hessia, Germany). Soil pH was measured in a slurry of 3 g soil in 9 ml dH_2O using a S220 SevenCompact pH meter (Mettler Toledo, Columbus OH, USA).

Data analyses

All statistical analyses were conducted in R version 3.1.3 (R Core Team 2015); linear mixed effects models were constructed using the nmle package (Pinheiro *et al.* 2015).

To investigate the effects of litter treatment, drought treatment, and their interaction on soil respiration, I used linear mixed effects models (*lme* function) with litter and drought treatments as fixed effects and time as a random effect. To account for the variation in room temperature in the greenhouse experiment, the

pots were blocked by average soil temperature, and block was included as an additional random effect in the models. To determine the significance of each term, I used nested models and compared the AICs to check for model improvement (Pinheiro & Bates 2000); where there was no significant difference in the model fit, the simpler model was chosen.

To assess the effects of litter and drought treatments on soil properties (total C and N, microbial biomass C) I used linear models. The full models included litter treatment, drought treatment, and their interaction. The models were simplified by sequentially dropping terms until a minimal adequate model was reached (Crawley 2007). For the drought treatment only, I also used this approach to investigate the effects of rewetting and the influence of plants on soil properties. The full model for the rewetting effect included litter treatment and sampling date (before or after rewetting) and their interaction; the full model for planting effect included litter treatment, the presence of plants (planted or unplanted) and their interaction

Results

Incubation experiments

Soil respiration in the incubation jars was extremely low before the start of treatments. Indeed, soil CO_2 efflux was verging on the detection limit of the infrared gas analyser (0.01 μmol m^{-2} s^{-1}). Soil CO_2 efflux increased by more than one order of magnitude upon addition of leaf litter to the jars in the double- and single-litter incubations (Fig. 1). The initial pulse of soil CO_2 at the time of litter application was

approximately twice as high in the double-litter compared to the single-litter treatment. However, there was no detectable change in the incubations that received the leachate treatment. During the drought phase of the experiment (20th March - 27th April), there was a noticeable decrease in soil CO_2 efflux, with a more rapid decline in the double-litter treatment compared to the single-litter incubations (Fig. 2).

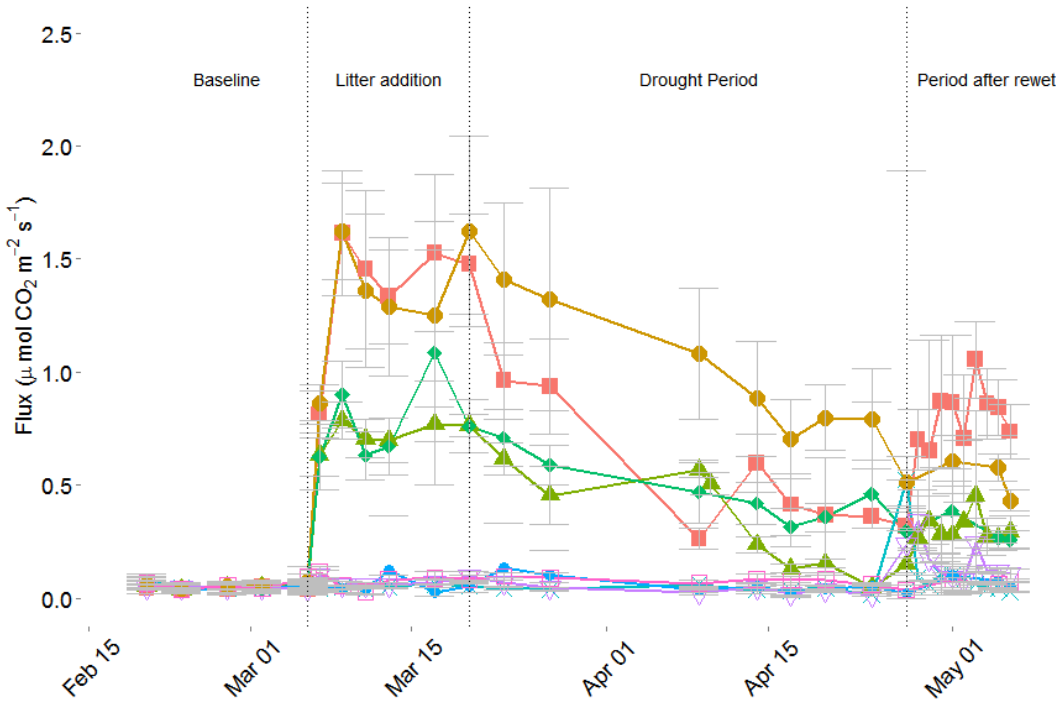


Figure 2. Soil CO_2 efflux from incubation jars during a three-month experiment with factorial drought and litter manipulation treatments, showing means and standard errors for *n* = 7 before rewetting and *n* = 3 or 4 after rewetting. Vertical dotted lines show the application of litter treatments, the start of the drought treatment and the time of rewetting; red squares = 2L drought, orange circles = 2L watered, lime green triangles = 1L drought, green diamonds = 1L watered, turquoise crosses = 0L drought, blue circles = 0L watered, purple triangles = leachate drought and pink squares = leachate watered, where 2L, 1L and 0L are double- single and no-litter treatments, respectively.

During three months of incubation, soil respiration differed significantly among litter and drought treatments; the final model also included a significant litter × drought interaction (χ^2 = 793.68, p < 0.001; Fig.3).

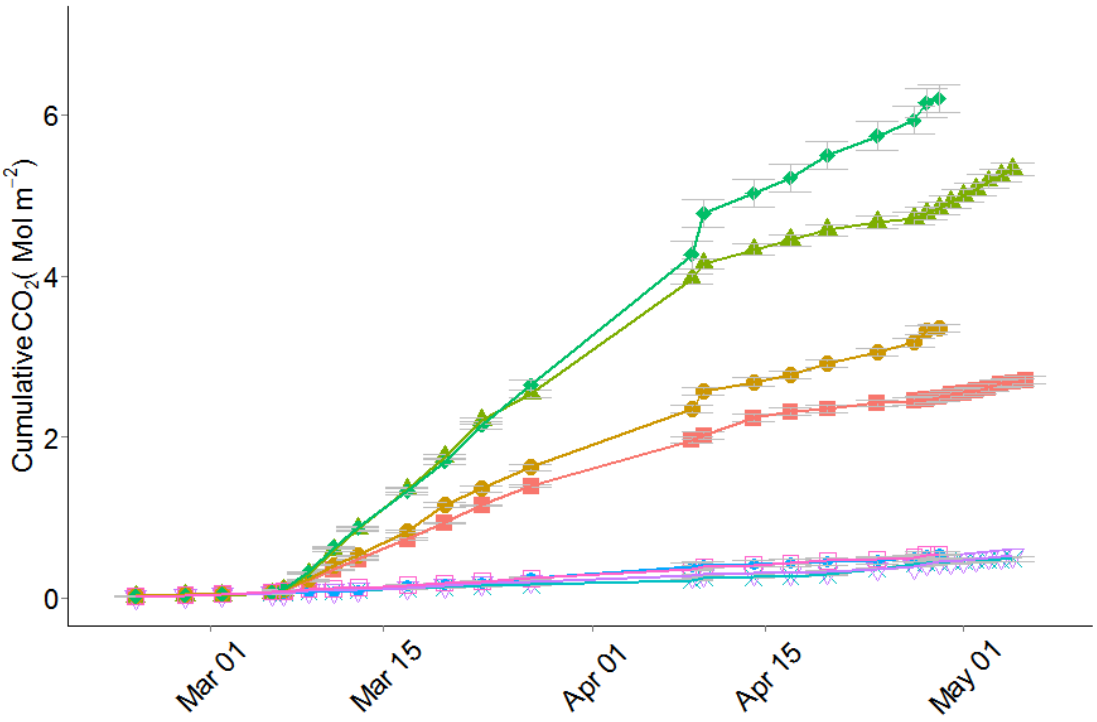


Figure 3. Cumulative soil CO_2 efflux from incubation jars during a three-month experiment with factorial drought and litter manipulation treatments, showing means and standard errors for *n* = 7 before rewetting and *n* = 3 or 4 after rewetting; lime green triangles = 2L drought, green diamonds = 2L watered, red squares = 1L drought, orange circles = 1L watered, turquoise crosses = 0L drought, blue circles = 0L watered, purple triangles = leachate drought and pink squares = leachate watered, where 2L, 1L and 0L are double- single and no-litter treatments, respectively.

To differentiate between the effects of litter treatments, drought treatments, and rewetting effects, I analysed the three periods of the experiment separately. During the two weeks following the start of litter treatments, soil respiration was significantly higher in jars with double-litter inputs compared to the other treatments (main treatment effect: χ^2 = 329.36 p < 0.001). During the drought period, soil respiration remained higher in the double- and single-litter treatments compared to

the no-litter and leachate treatments. However, the decrease in soil respiration with drought was greatest in the double-litter treatment and the final model included a significant litter × drought interaction (χ^2 = 491.72 p < 0.001; Fig. 4).

Similarly, the increase in soil respiration after rewetting was greatest in the double-litter treatment and the final model included a significant litter × drought interaction (χ^2 = 223.94 p < 0.001; Fig. 4).

Total soil carbon and nitrogen content at the end of the drought period were significantly higher in the double-litter incubations compared to the other litter treatments (soil C: $F_{3,24}$ = 4.21, p = 0.016; soil N: $F_{3,24}$ = 3.86, p = 0.022; Figs. 4 and 5, respectively). Despite the differences in soil respiration, microbial biomass C was similar among drought and litter treatments both before and after rewetting (Fig. 7).

In the incubation jars with litter additions, there was no effect of drought on litter decomposition but relative mass loss was greater in the single-litter compared to the double-litter treatments ($F_{2,14}$ = 5.44, p = 0.018; Fig. 8).

In the incubations subjected to drought, there were no significant differences in soil C, N or microbial biomass before and after rewetting in any of the litter treatments.

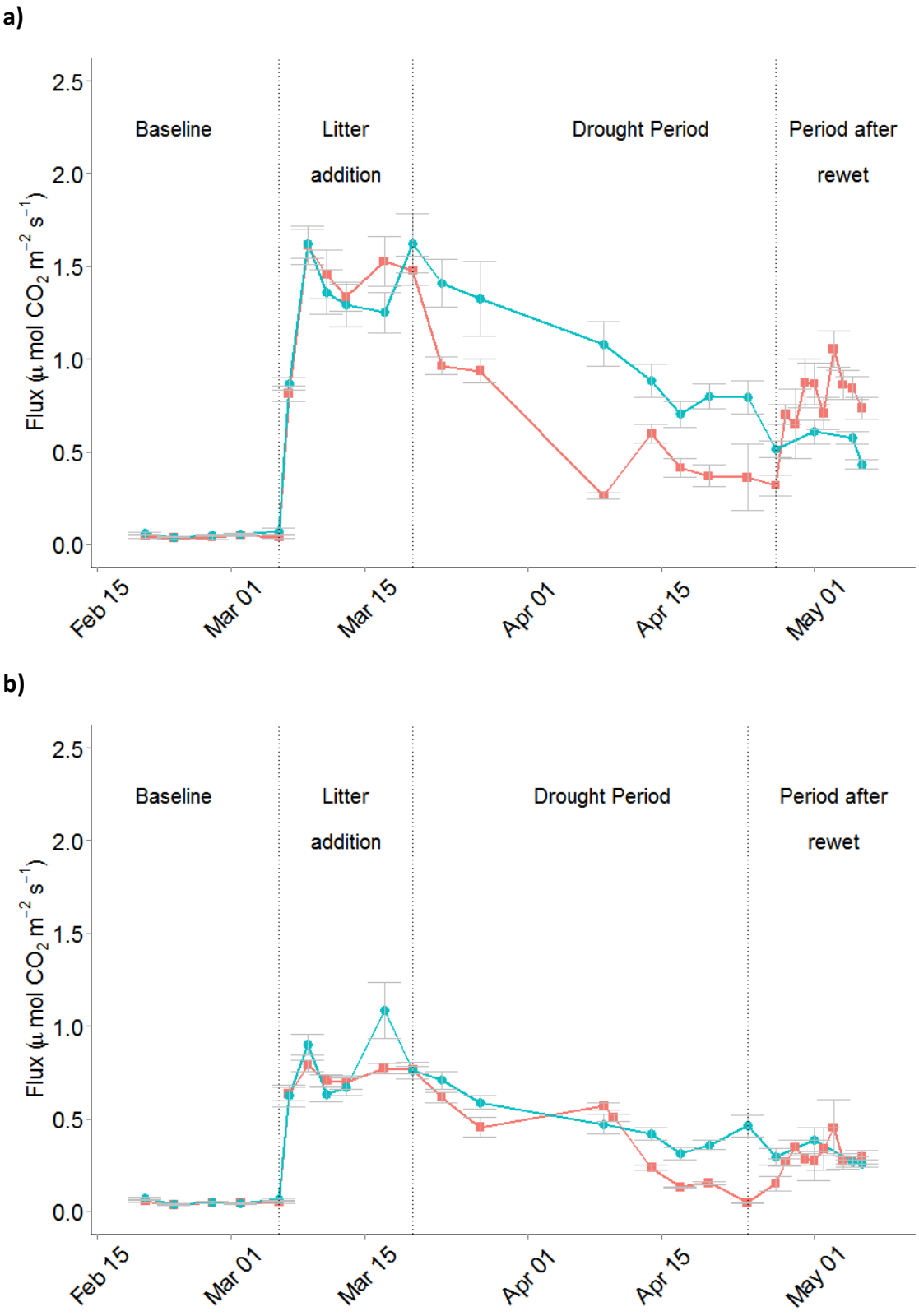


Figure 4 Differences in soil CO_2 efflux between drought treatments for incubation jars with **a)** double-litter inputs and **b)** single-litter inputs during a three-month experiment, showing means and standard errors for n = 7 before rewetting and n = 3 or 4 after rewetting; vertical dotted lines show the application of litter treatments, the start of the drought treatment and the time of rewetting, respectively; red squares indicate the drought treatment and blue circles are watered jars.

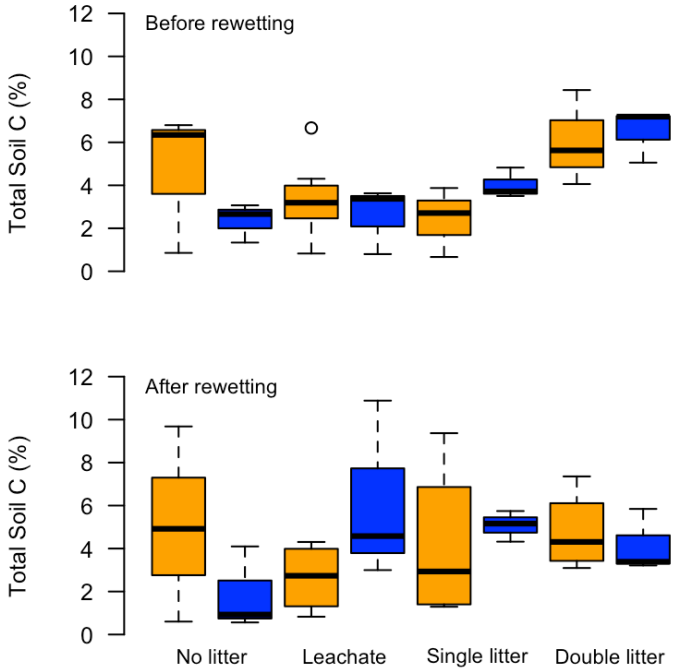


Figure 5 Soil carbon content in droughted (orange) and watered (blue) incubations with different litter addition treatments, measured before rewetting and after rewetting; boxplots show 1st and 3rd quartiles and 95% confidence interval of medians for *n* = 3 or 4.

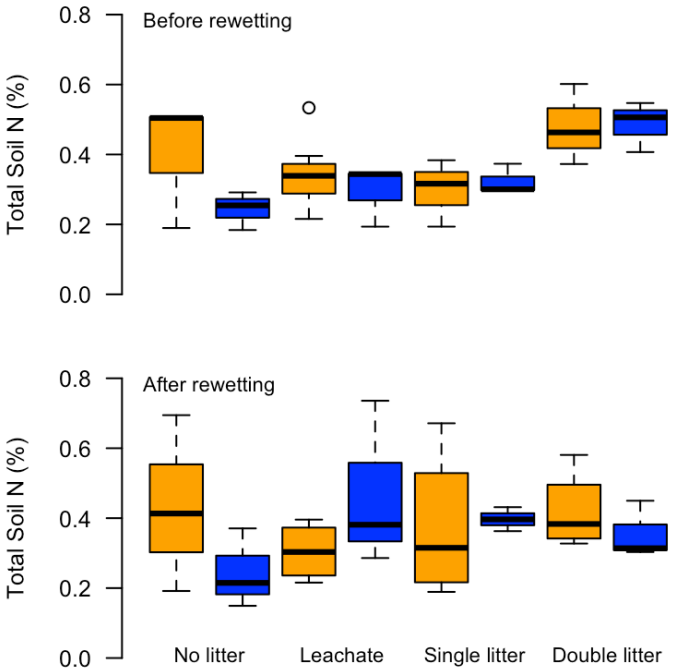


Figure 6 Soil nitrogen content in droughted (orange) and watered (blue) incubations with different litter addition treatments, measured before and after rewetting; boxplots show 1st and 3rd quartiles and 95% confidence interval of medians for *n* = 3 or 4.

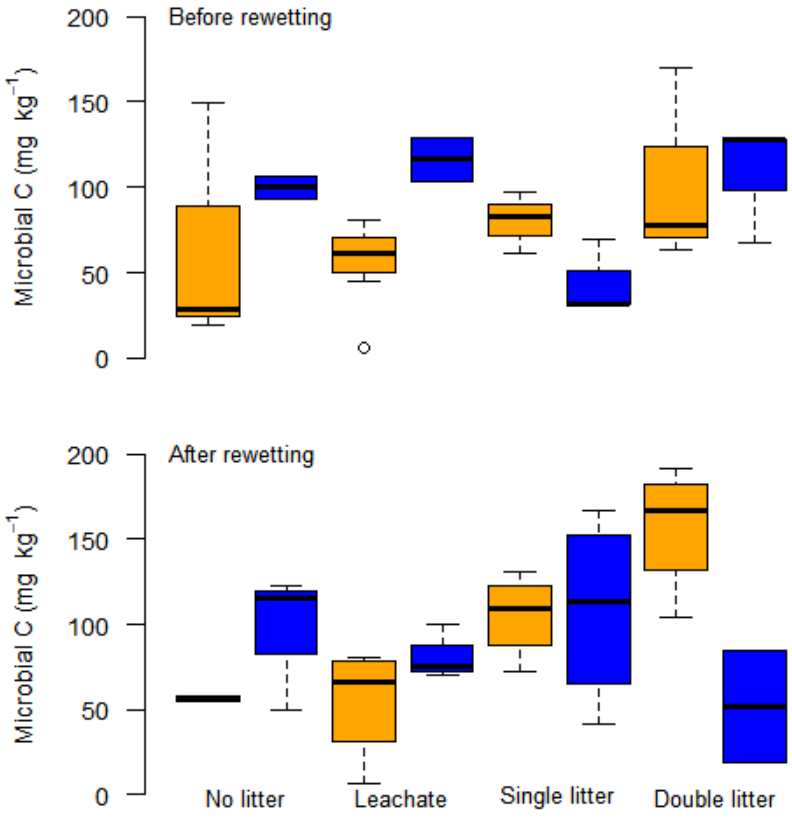


Figure 7. Soil microbial biomass carbon in droughted (orange) and watered (blue) incubations with different litter addition treatments, measured before rewetting and after rewetting; boxplots show 1st and 3rd quartiles and 95% confidence interval of medians for *n* = 3 or 4.

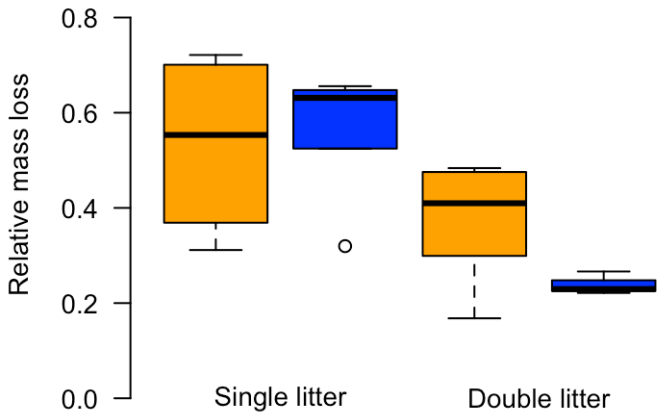


Figure 8. Relative mass loss of litter at the end of a three-month incubation experiment in droughted (orange) and watered (blue) jars with different litter addition treatments; boxplots show 1st and 3rd quartiles and 95% confidence interval of medians for *n* = 3-5.

Greenhouse experiment

Similar to the incubation experiments, there were four distinct periods in the pattern of soil respiration during the course of the study: 1) a one-week establishment period before the application of the litter treatments; 2) a two-week pre-drought period, in which the largest litter treatment effects were observed; 3) the main drought period in which soil CO_2 efflux declined steadily in most drought pots; and 4) the rewetting period, with highly variable soil CO_2 efflux (Fig. 9).

Soil respiration

Overall, soil CO_2 efflux was significantly affected by litter additions and drought treatments relative to controls (χ^2 = 175.35, p < 0.001). Soil respiration was noticeably lower in the drought treatment compared to the watered pots and soil respiration also tended to increase with the amount of litter added (Fig. 8) but there was no significant litter × drought interaction (χ^2 = 0.981, p = 0.7542).

In the planted pots, I observed a distinct peak in soil CO_2 efflux in response to the application of litter treatments in the second week of the study (Fig. 11). Over the remaining study period, soil CO_2 efflux in the watered pots was consistently higher in the double-litter treatment (mean: 2.33 umol m^{-2} s^{-1}) compared to the no-litter treatment (mean: 1.62 umol m^{-2} s^{-1}).

In pots without trees, there was a similar peak in respiration after litter addition and soil respiration was also highest in the double-litter treatments but the effects of drought were less clear because soil CO_2 efflux was lower in the watered pots than in the drought treatment (Figure 12).

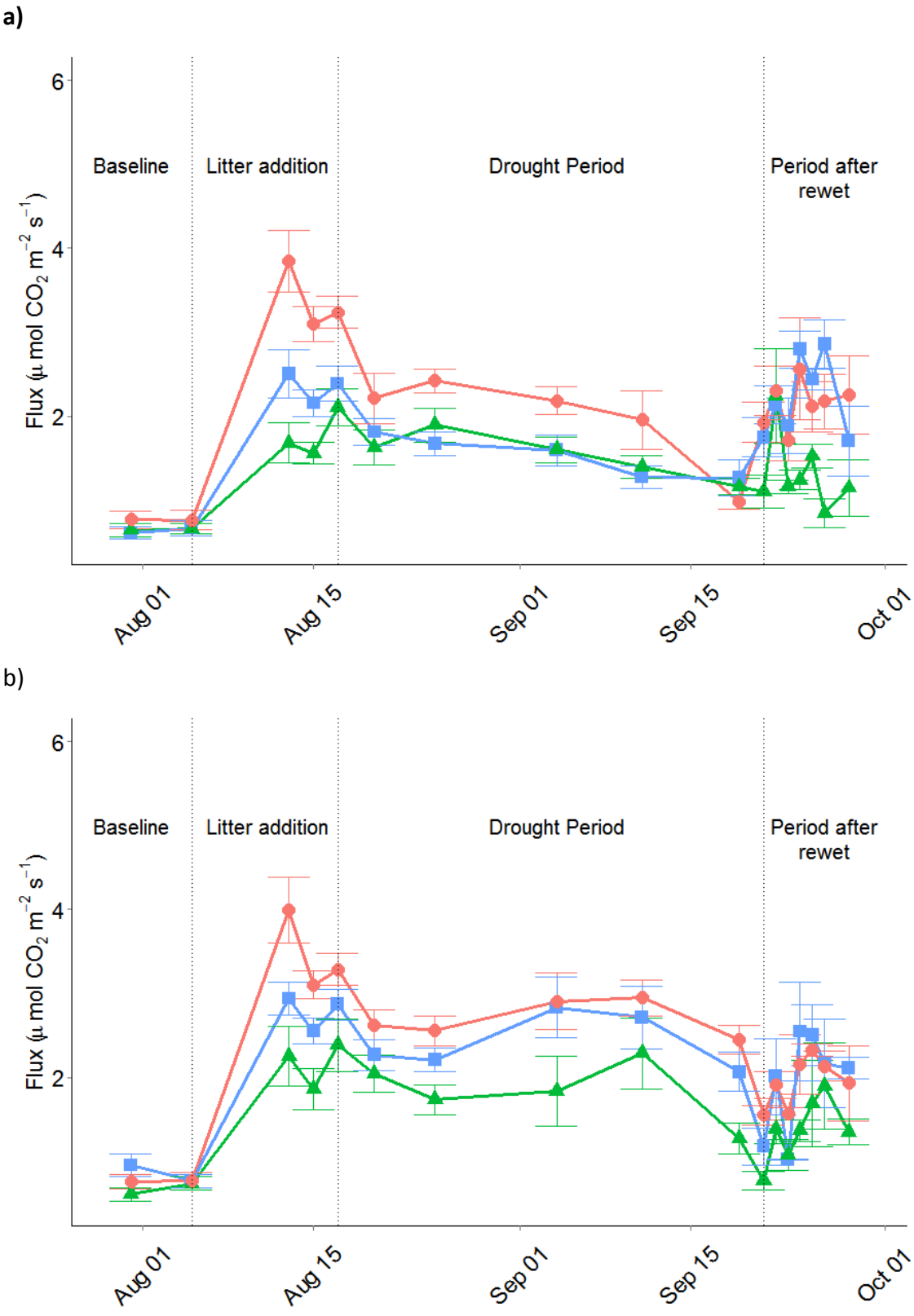
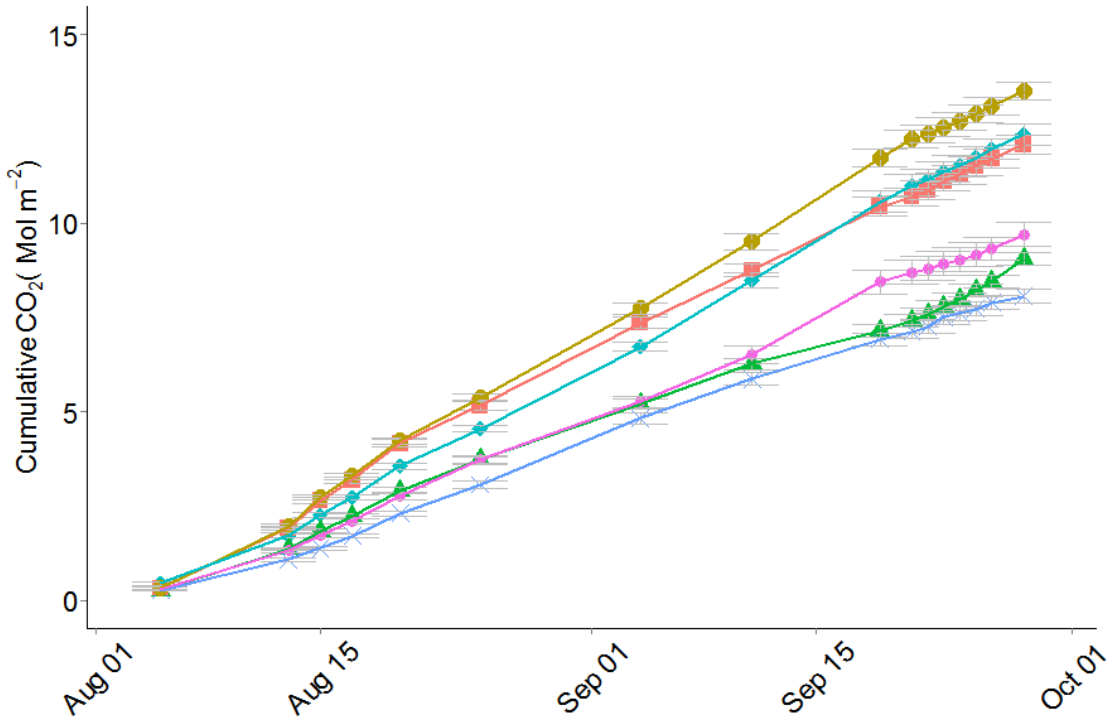


Figure 9 Soil respiration in pots planted with poplar cuttings with different litter treatments and **a)** subjected to a drought treatment or **b)** watered daily, showing the distinct periods during the experiment; means and standard errors are given for *n* = 12; red circles are double-litter treatments, blue squares are single-litter treatments and green triangles are no-litter treatments.

a)



b)

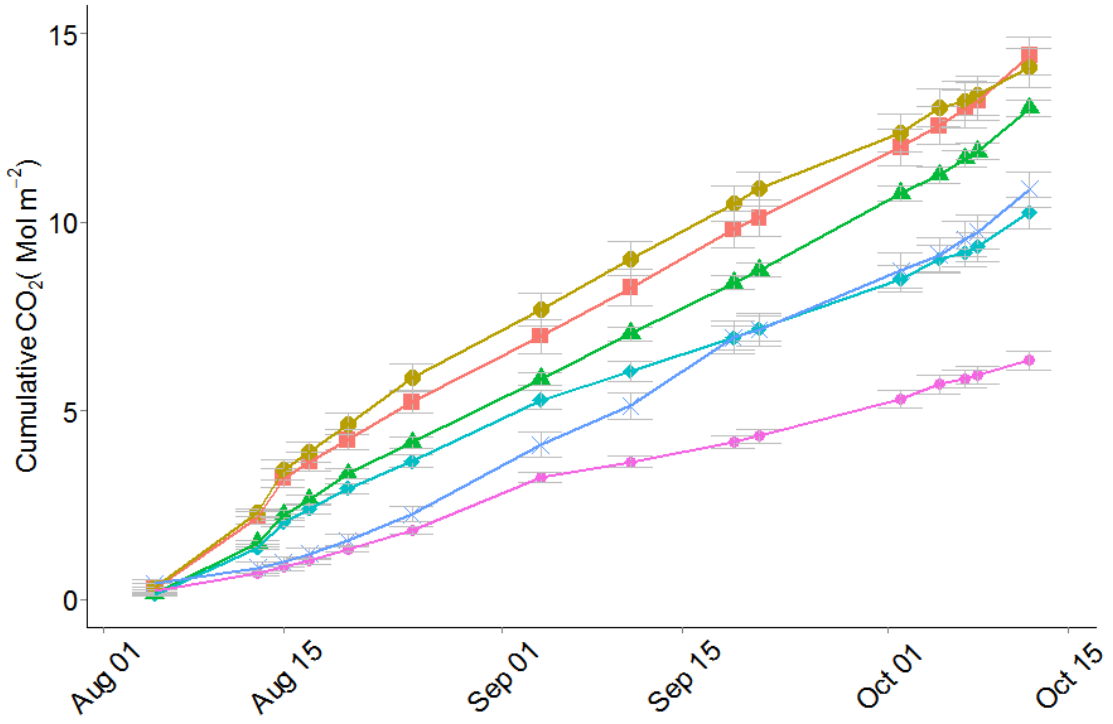


Figure 10 Cumulative soil CO_2 efflux in **a)** pots planted with poplar seedlings and **b)** unplanted pots during a three-month greenhouse experiment with factorial drought and litter manipulation treatments; means and standard errors are given for *n* = 12 and *n* = 4 for planted and unplanted pots, respectively; yellow circles = 2L watered, red squares = 2L drought, turquoise diamonds = 1L watered, green triangles = 1L drought, pink circles = 0L watered and blue crosses = 0L drought, where 2L, 1L and 0L are double- single and no-litter treatments, respectively.

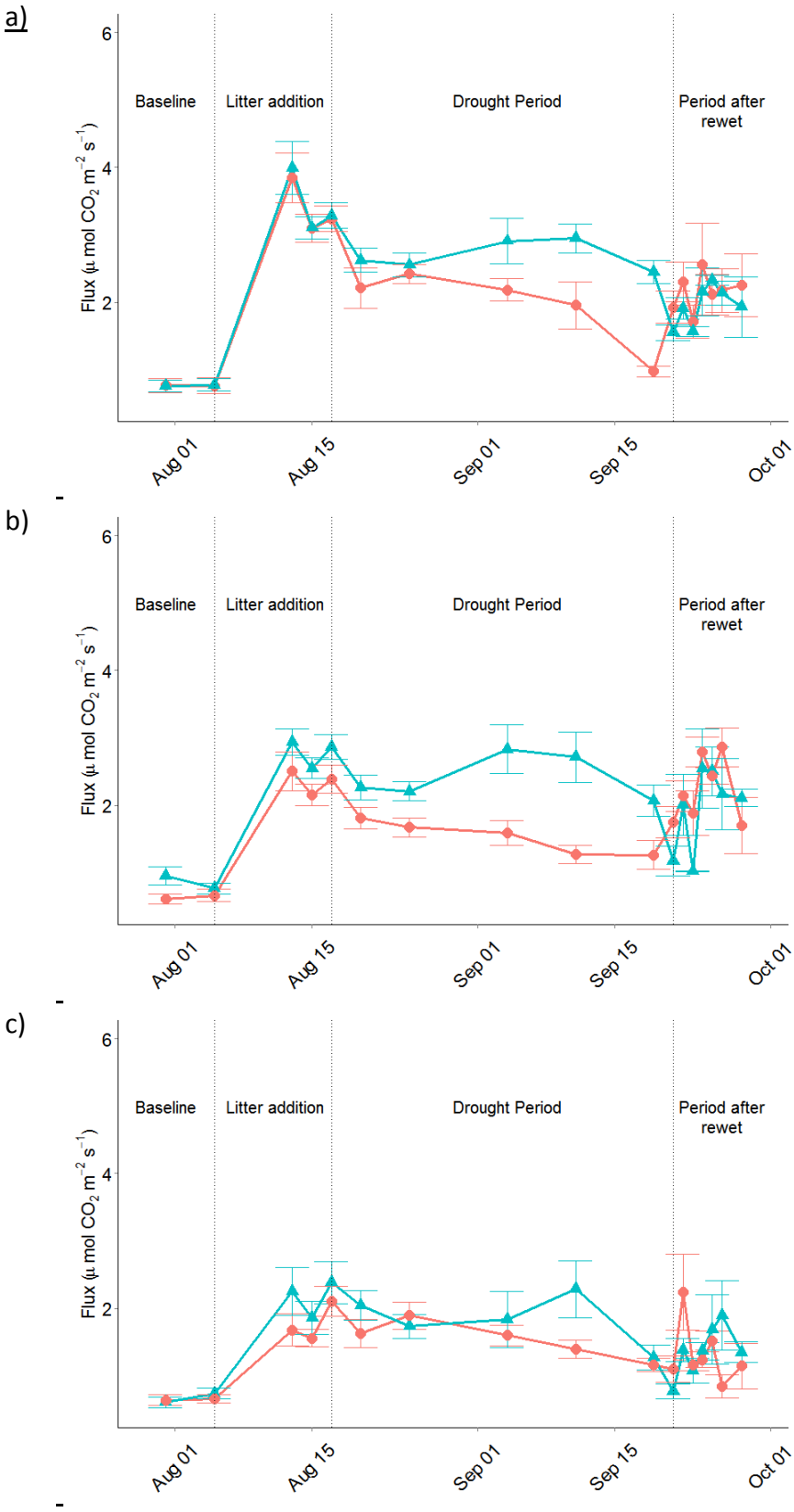


Figure 11 Soil respiration in pots planted with poplar cuttings and with **a)** double-litter, **b)** single-litter and **c)** no-litter treatments; blue triangles indicate watered treatments and red circles show droughted pots; means and standard errors are shown for *n* = 12.

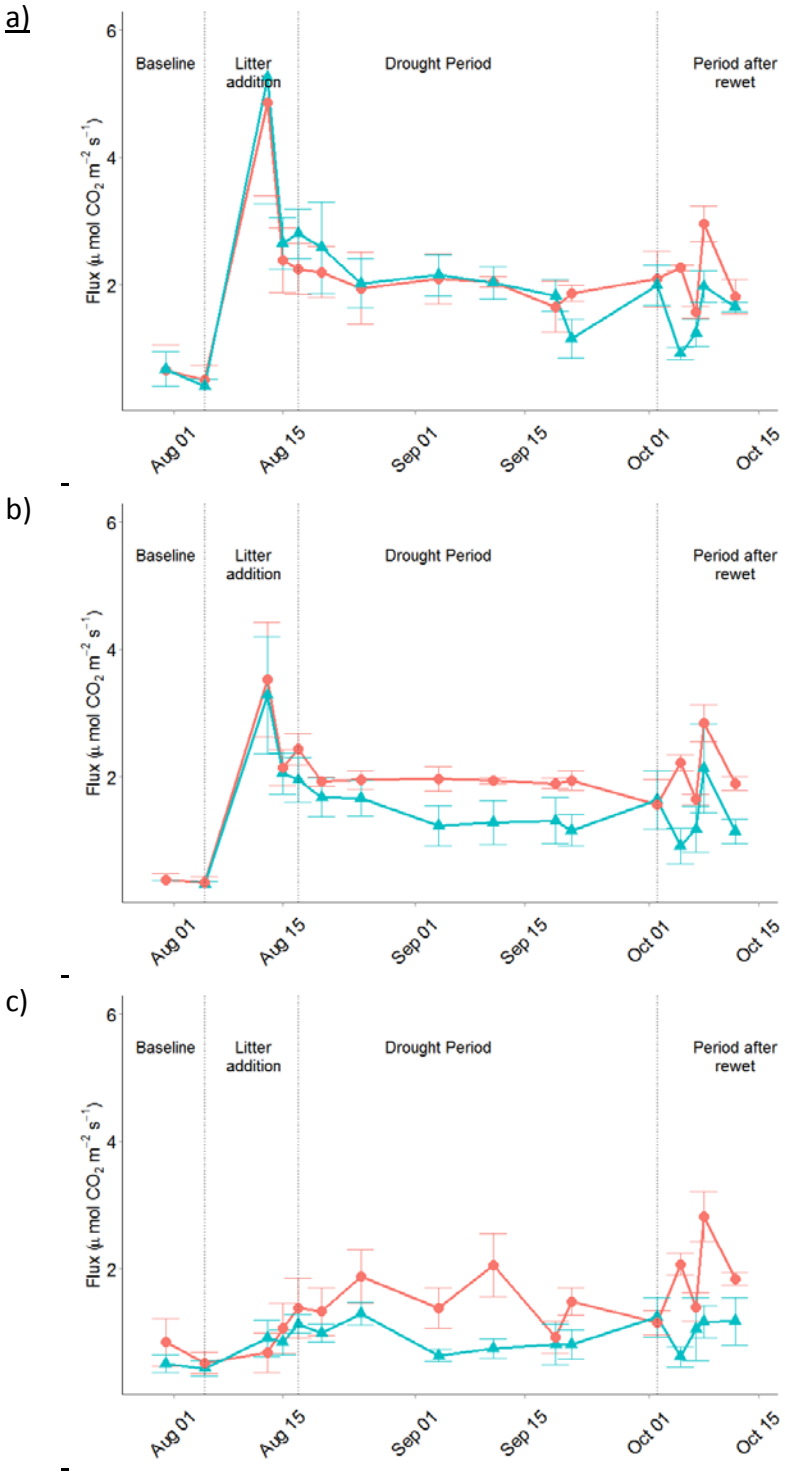


Figure 12 Soil respiration in unplanted pots with **a)** double-litter, **b)** single-litter and **c)** no-litter treatments; blue triangles indicate non-drought treatments and red circles show droughted pots; means and standard errors are shown for *n* = 4.

Effects of drought and litter additions on soil respiration

For the drought period, the best model included the litter and drought treatments but not their interaction (χ^2 = 89.299, p < 0.001); overall, the litter treatments explained more of the variation in soil CO_2 efflux than drought (litter treatment effect: χ^2 = 10.7668 p < 0.001; Fig. 11).

In the period immediately after the drought, when the birch effect is typically observed, only the litter treatments had a significant effect on soil CO_2 efflux (χ^2 = 38.089, p < 0.001). Surprisingly, including the drought treatment did not improve the model fit (χ^2 = 1.3438; p = 0.2464). A comparison of planted and unplanted pots after rewetting showed the presence of trees and the litter treatments had a significant effect on soil CO_2 efflux (χ^2 = 33.05, p < 0.001), but there were no interactions between any of the treatments.

Treatment effects on soil microbial biomass and soil chemistry

In pots planted with poplar cuttings, there were no differences in soil C, soil N or microbial biomass C at the peak of the drought period. However, after rewetting, total soil C was higher in drought treatments compared to watered pots regardless of litter treatment ($F_{3,18}$ = 3.93, p = 0.025; Fig. 13) and there was a significant litter x drought interaction on total soil N ($F_{5,16}$ = 5.11; p = 0.005); total N in the soil after rewetting was highest in the drought treatments with single-litter addition (Fig. 14).

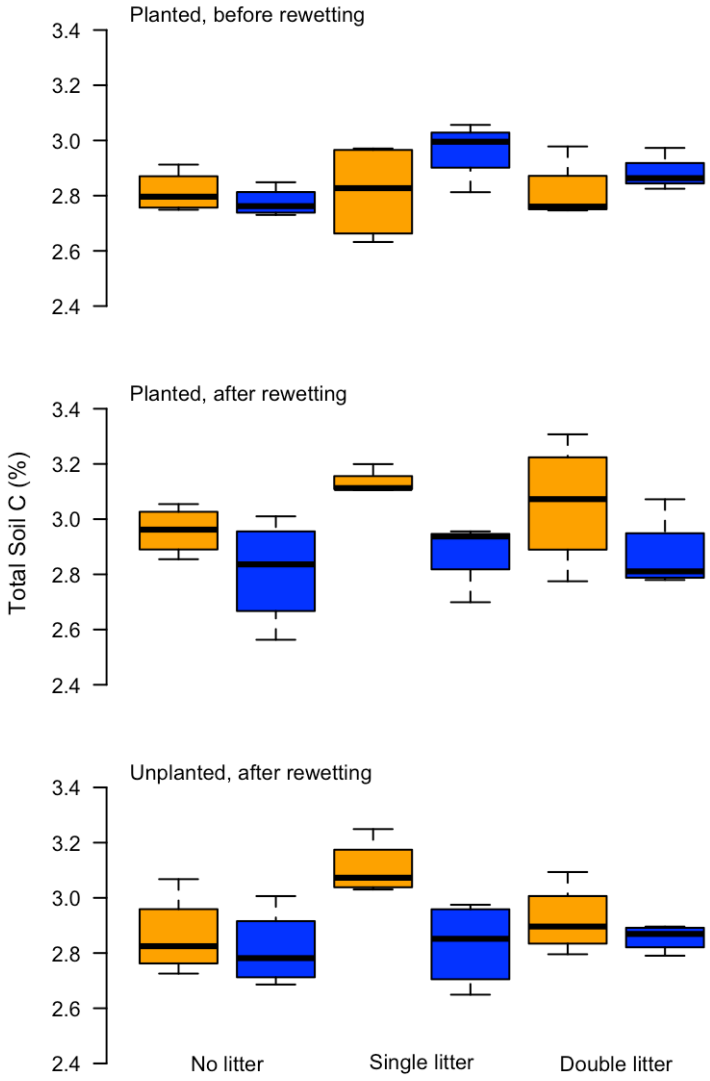


Figure 13. Total soil carbon in droughted (orange) and watered (blue) pots with different litter addition treatments, measured at the peak of the drought period (before rewetting, planted pots only) and after rewetting at the end of the experiment; boxplots show 1st and 3rd quartiles and 95% confidence interval of medians for n = 12 and n = 4 for planted and unplanted pots, respectively.

In the unplanted pots, total soil C after rewetting was best described by the model including the interaction of drought and litter treatments ($F_{5,18}$ = 3.011; p = 0.038); total soil C was higher in drought treatments compared to watered pots and the difference was greatest with single-litter addition (Fig. 13). Soil microbial biomass C in unplanted pots was unaffected by litter addition but was marginally lower in

drought treatments compared to watered pots at the end of the study (main treatment effect: $F_{3,20} = 2.83$; $p = 0.064$; Fig. 15).

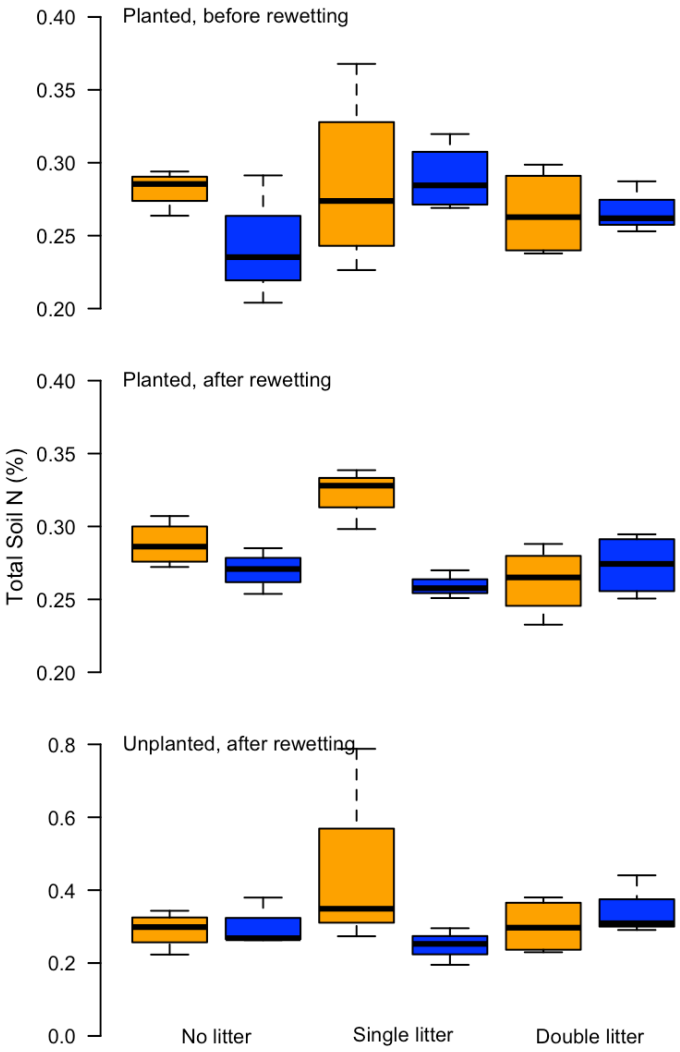


Figure 14. Total soil nitrogen in droughted (orange) and watered (blue) incubations with different litter addition treatments, measured at the peak of the drought period (before rewetting, planted pots only) and after rewetting at the end of the experiment; boxplots show 1st and 3rd quartiles and 95% confidence interval of medians for $n = 12$ and $n = 4$ for planted and unplanted pots, respectively.

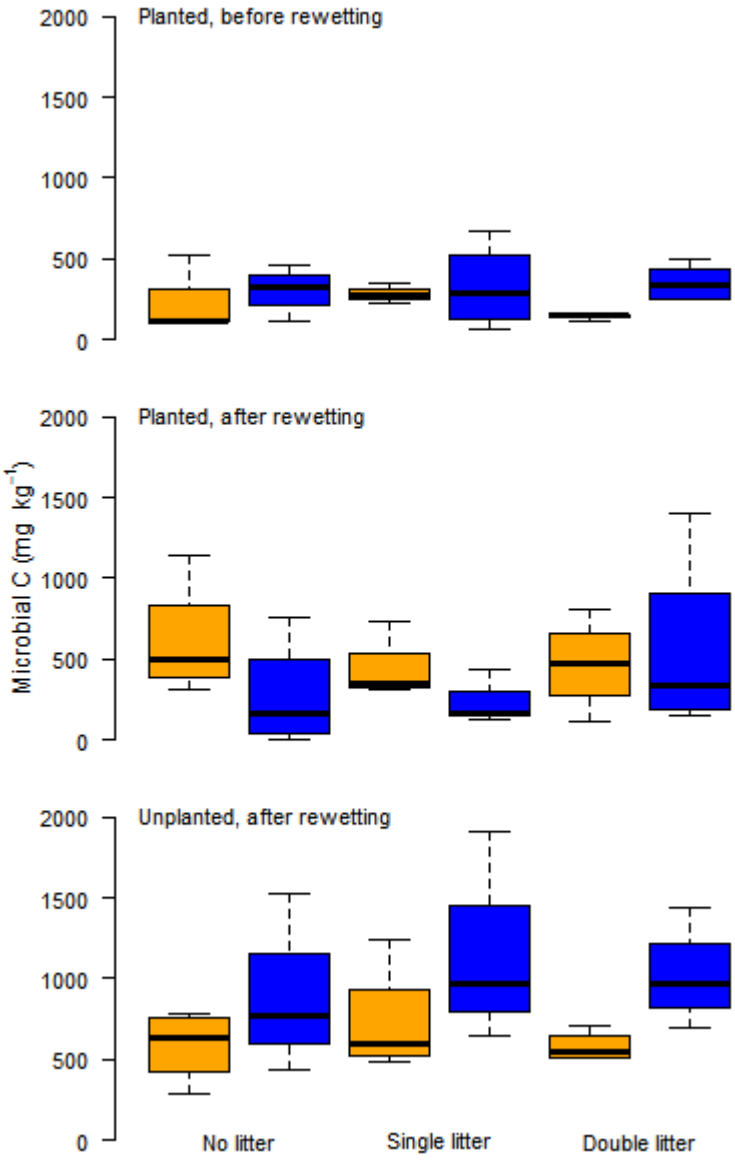


Figure 15. Microbial biomass carbon in droughted (orange) and watered (blue) pots with different litter addition treatments, measured at the peak of the drought period (before rewetting, planted pots only) and after rewetting at the end of the experiment; boxplots show 1st and 3rd quartiles and 95% confidence interval of medians for n = 12 and n = 4 for planted and unplanted pots, respectively.

In the pots subjected to drought, there was a significant increase in total soil C after rewetting ($F_{3,20}$ = 3.29, p = 0.04), and although there was no significant drought x litter interaction, the most pronounced increase was observed in the single-litter treatment (Fig. 15). Microbial biomass carbon was greater after rewetting regardless of litter treatment or the presence of plants ($F_{3,17}$ = 3.22, p = 0.049; Fig. 16).

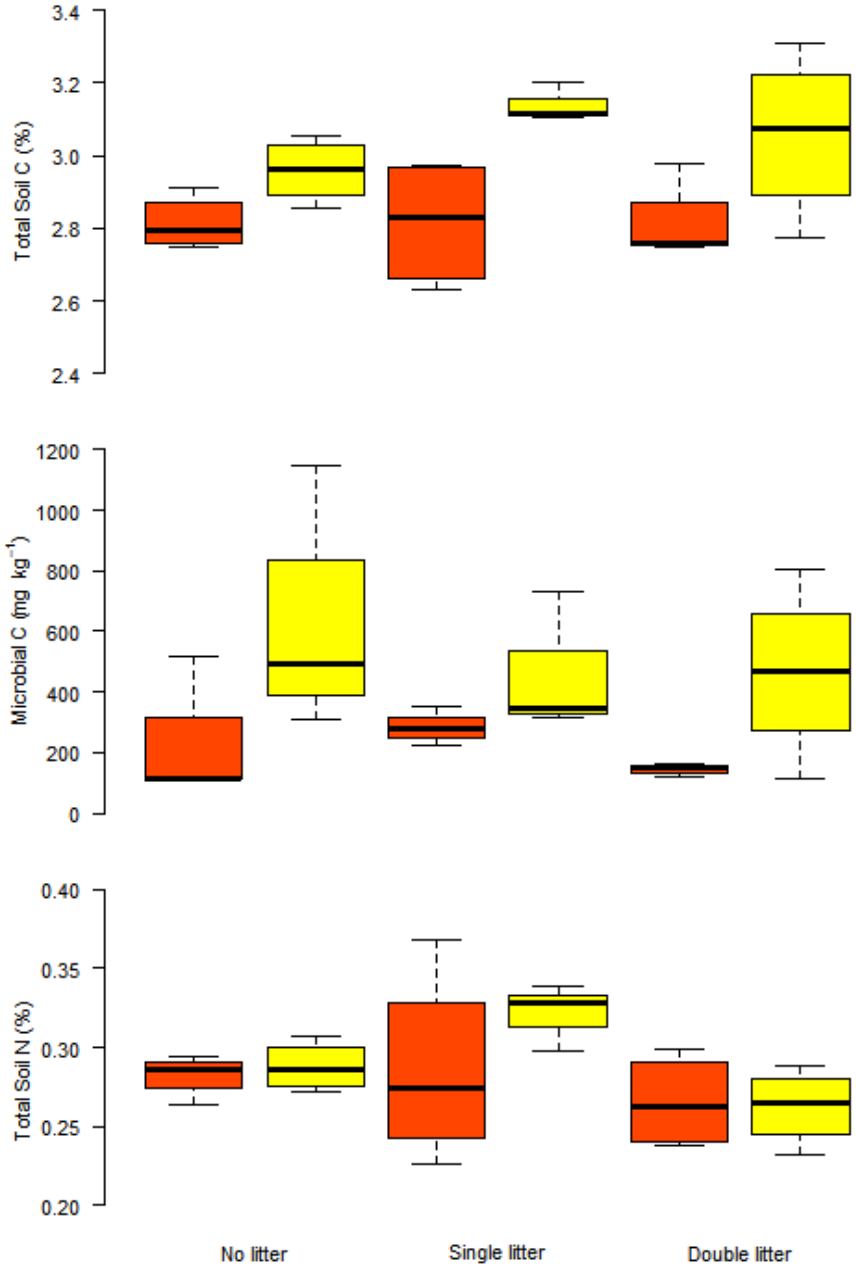


Figure 16. Soil carbon and nitrogen content and microbial biomass carbon in droughted pots planted with polar cuttings and with different litter addition treatments, measured at the peak of the drought period (red boxes) and after rewetting at the end of the experiment (yellow boxes); boxplots show 1st and 3rd quartiles and 95% confidence interval of medians for *n* = 12.

Discussion

The main goal of my experiments was to investigate the possible interaction between the Birch effect and the priming effect and their influence on soil respiration after drought. The results from the two different experiments tell different stories: in the incubation experiment, I only observed a response to drought in the treatments with added litter but there was little or no response to drought in the leachate and no-litter treatments. On the other hand, the results from the greenhouse show a strong drought response in planted pots but little interaction between litter and drought treatments. Extra litter additions increased respiration whereas the presence of poplar trees decreased the size of the Birch effect.

Incubation experiment

In my incubation experiments, the litter additions had an overall greater effect on soil respiration and soil properties than the drought treatment. The soil I used for the incubations was an artificial commercial 'topsoil', with very poor soil structure and very low biologically available carbon. As soil microorganisms are generally carbon-limited (Smith & Paul 1990), the litter additions were probably necessary for microbial growth. Consequently, soil nitrogen and carbon content was higher in the double-litter treatment before rewetting, regardless of whether the incubations had been subjected to drought or kept watered.

The extremely low respiration rates in the incubation jars at the start of the experiment indicates that the soil organic matter content was insufficient to sustain much microbial activity. Consequently, it is likely that the pulse of CO_2 measured after the application of the litter treatments was largely due to soil microbial growth

and the rapid colonization by microorganisms (fungal mycelia and bacteria) of the added litter (Romani *et al.* 2006; Steffen *et al.* 2007; Snajdr *et al.* 2008). This is supported by the larger peak in response to the double-litter addition, which was approximately twice as high as the single-litter treatment. Correspondingly, soil respiration in the double-litter incubations declined much more rapidly after the start of drought, as water becomes limiting and the litter on the soil surface dried out rapidly (Clein and Schimel 1994; Berg *et al.* 1998). Leaching of organic solutes contributes to litter decomposition (Cotrufo *et al.* 2015) but the negligible effects of the leachate treatments on CO_2 efflux suggest that the mineralization of dissolved organic carbon from litter and artificial leachates did not contribute substantially to the observed microbial respiration in my study.

Although the drought imposed on the incubations was severe, I did not observe the characteristic peak in soil CO_2 efflux during the first three to four days upon rewetting that is usually associated with the Birch effect (Kim *et al.* 2012; Fig. 3). This is likely a result of the lack of soil structure in the artificial 'commercial topsoil', as the presence of soil aggregates (Denef *et al.* 2001) and fine particles to which organic solutes associate (Lundquist *et al.* 1999; Wang *et al.* 2003; Casals *et al.* 2009) are thought to play a key role in the Birch effect. Instead, respiration increased steadily over the course of a week after rewetting. Three different mechanisms could explain the pattern in soil CO_2 efflux after rewetting: i) leaching of labile components from the litter (Hagedorn and Machewitz 2007), ii) further microbial colonisation of the remaining litter, and iii) soil microbial growth and recovery from dormancy (Schimel *et al.* 2007). In my study, the peak in respiration in the droughted double-litter jars after rewetting is probably a combination of the three mechanisms, whereby a larger

amount of labile carbon from the remaining litter was available to microbial decomposers, which fuelled a sudden increase in microbial growth and colonisation of the litter. This explains the higher microbial biomass after rewetting (Fig. 6) and the greater relative mass loss of litter in the double-litter drought treatments (Fig. 7).

Unexpectedly, the leachate treatment appeared to have no effect on soil respiration and a negligible effect on microbial biomass. Tree litter leachates are thought to be a major source of labile C for soil microbes (Kalbitz *et al.* 2000; Don and Kalbitz 2005). However, some studies have challenged this assumption, suggesting that only a fraction of the carbon in leachates is immediately available to soil microbes (Qualls and Haines 1992; De Troyer *et al.* 2011). The easily-degradable carbon in leachate can be consumed in a matter of hours (De Troyer *et al.* 2011), whereas recalcitrant solutes take a long time to decompose (Qualls and Haines 1992). In my incubations, soil respiration in the leachate treatments was very low (Figs. 1 and 2) which supports the notion that only a small fraction of the carbon was available for microbial use. In addition, I took soil CO_2 measurements at least one day and up to four days after applying leachates. Hence, given the rapid cycling of the labile carbon from leachate (De Troyer *et al.* 2011), any potential microbial respiration in response to leachate addition could have happened very rapidly and not shown up in my measurements.

Greenhouse experiment

Overall the litter treatments had a greater effect on soil respiration than rewetting. It is important to note that soil respiration in the greenhouse experiment was strongly influenced by litter treatment, even though litter was removed from the soil collars

before measurements. This approach has previously been used in field studies using litter manipulation treatments because it excludes variation due to decomposition of different amounts of litter on the soil surface (Sayer *et al.* 2007), which allowed me to observe belowground responses to litter and drought treatments.

Once the drought treatment was applied, there was a notable rapid drop in soil respiration in the drought treatments with poplar cuttings (Fig. 10), whereas the unplanted pots maintained high respiration rates throughout the drought period (Fig. 11). The drought treatment was more effective in planted pots because plant water uptake (and transpiration) will have contributed to rapid desiccation of the soil (Jackson *et al.* 2000). To partly compensate for this in the unplanted pots, I extended the drought period for an extra week until they reached the same soil moisture content as the planted pots.

I only analysed the data from unplanted pots subjected to the drought treatment because the watered pots without poplar cuttings quickly became waterlogged. At the start of the experiment, I decided to add the same quantity of water to all the watered pots because it was not feasible to individually determine water holding capacity in all 96 pots. I calculated the required amount of water for the planted pots but this proved to be too much for the unplanted pots due to the lack of water uptake by plants and the soil became waterlogged. Soil waterlogging is likely to have contributed to the lower respiration rates observed in the unplanted watered pots (Fig. 11), as it reduces air volume in soil pores, which can suppress microbial activity and gas diffusion (Linn and Doran 1984).

The drought treatment in the planted pots was highly effective; I observed substantial leaf loss from the poplars, which highlights the importance of considering the effect of additional litter inputs to the soil during severe drought. Indeed, the lack of clear differences in respiration rates between litter treatments after rewetting in the droughted pots could conceivably be a result of litterfall from the stressed plants. Even though I endeavoured to remove the extra litter from the pots regularly, it could have contributed nutrients and carbon to the soil before it was removed and may explain the large increase in total soil carbon in the single-litter treatment after rewetting (Fig. 12).

I rewet the soil shortly before the plants had reached permanent wilting point to maximise the drought conditions but avoid killing the poplars. As the poplar trees were placed under severe stress, it is likely that significant fine-root mortality occurred within the pots. This would have provided another source of carbon and nutrients into the soil after rewetting (Jones *et al.* 2004). The additional carbon and nitrogen from plant litter probably decreased the difference in microbial biomass carbon among the litter treatments by providing labile C for immediate use by soil microbes. It could also partly explain the higher carbon and nitrogen content after rewetting in drought treatment compared to the watered treatments (Fig. 12-14).

Many soils have a tendency to crack when undergoing drought. This phenomenon is normally exacerbated where the soil is contact with a solid object, such as the soil collars and the edge of pots. I did not measure soil respiration when the soil inside the collars was badly cracked, as the cracking would have changed the CO_2 efflux in two ways; first the cracking increases the surface area of the soil while at the same

time exposing lower depths to the air; this exposure can increase the amount of CO_2 released from the soil (Rochette and Hutchinson 2005). Secondly, the cracks in the soil can potentially compromise the seal between the chamber and the soil collars during measurements. If the system is not properly sealed, air will be exchanged with the atmosphere during the measurements and the accumulation of the CO_2 is disrupted.

After the rewetting event, there was a much smaller CO_2 efflux response in the pots planted with poplar cuttings compared to those without plants, even though the drought had a greater effect on soil respiration in the planted pots. Soil respiration in the planted pots recovered to values similar to those observed in the non-drought treatments but did not exceed them (Fig. 10), which would be expected with Birch effects. This lack of a large peak in soil CO_2 efflux differs from the majority of the literature on drying and rewetting effects on soil, as many of the experiments are conducted without plants (Mikha *et al.* 2005; Miller *et al.* 2005; Wu & Brookes 2005; Beare *et al.* 2009; Xiang *et al.* 2008). This change in the pattern of soil CO_2 efflux with the presence of plants is most likely due to the complex interaction between soil microbes and root products, in particular root exudates. Although a full investigation of these interactions was beyond the scope of my experiments, it presents an interesting avenue for future research. Plant-soil interactions are central to a large number of important biogeochemical processes and they are also influenced by properties of both the soil and the roots (Bouma & Bryla 2000). To further complicate matters, interactions between the rhizosphere and the bulk soil are not uniform throughout the soil, as root growth varies greatly in space and time.

The comparison of the drought treatments before and after rewetting revealed a sizeable increase in microbial biomass carbon suggesting an increase in microbial growth upon rewetting, possibly as a result of dead root material (discussed above). The higher soil carbon content in droughted pots after rewetting is probably largely accounted for by this microbial growth (Fig. 15). Thus, although I did not observe the clear peak in soil CO_2 efflux associated with typical Birch effects, my results indicate that the more gradual increase in soil respiration after rewetting is a result of increased microbial biomass and activity, which is sustained by root carbon inputs.

Conclusions

I found evidence to suggest that the presence of plant roots moderated the release of CO_2 from the soil after severe drought and that plant roots reduced the amount of CO_2 potentially released through priming effects in response to increased litter inputs However, I was unable to determine conclusively whether the observed responses were indeed Birch effects and priming effects. Further work is required to determine whether Birch effects and priming effects have an interactive effect on soil carbon release. There are also many possibilities to take the work beyond the initial scope of these experiments; interesting future avenues for research could include the effect of tree species, the influence of soil type, differences in the length and severity of drought, repeated rewetting and drying cycles, and importantly, scaling up experiments to simulate field conditions. This last point is critical, as both priming and Birch effects could result in substantial release of CO_2 from soils after

drought, and we need to understand how different mechanisms and feedbacks affect one another to predict carbon dynamics in ecosystems under global change.

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Appendix

a)

b)

Figure A. Photographs of the experiments described in this thesis a) jars used for the incubation experiment with and without lids and different drought treatments b) some of the poplar saplings in the greenhouse pot experiment.

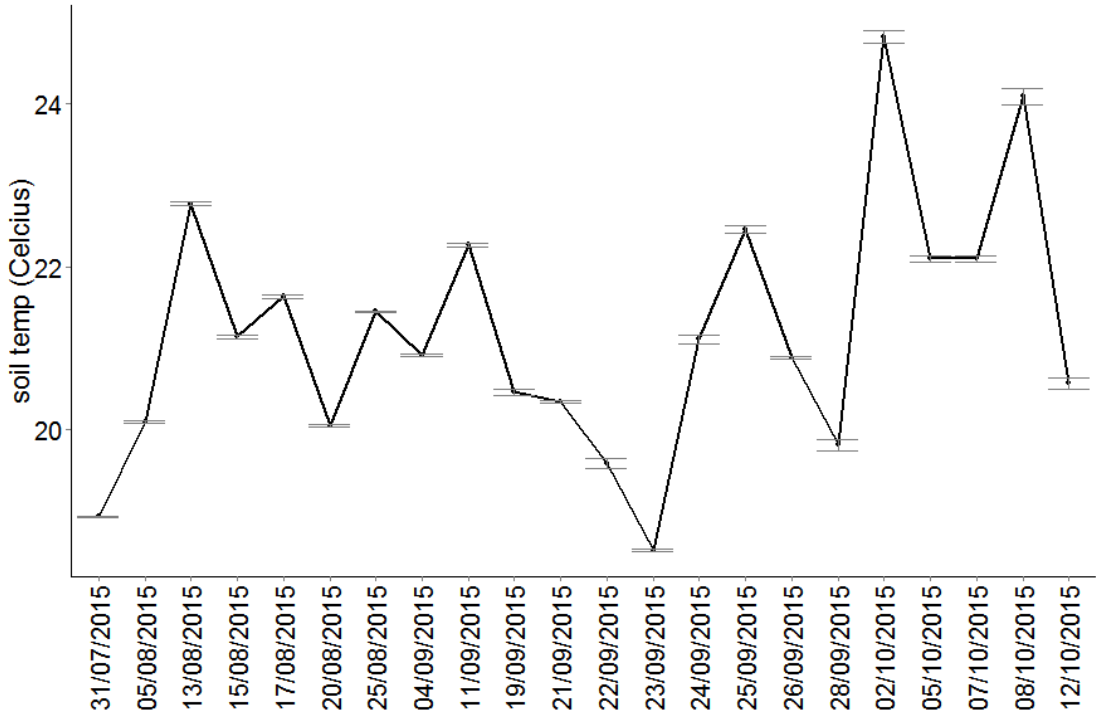

Figure B. Average soil temperatures in pots in the greenhouse experiment, showing standard errors of means for *n* = 96.

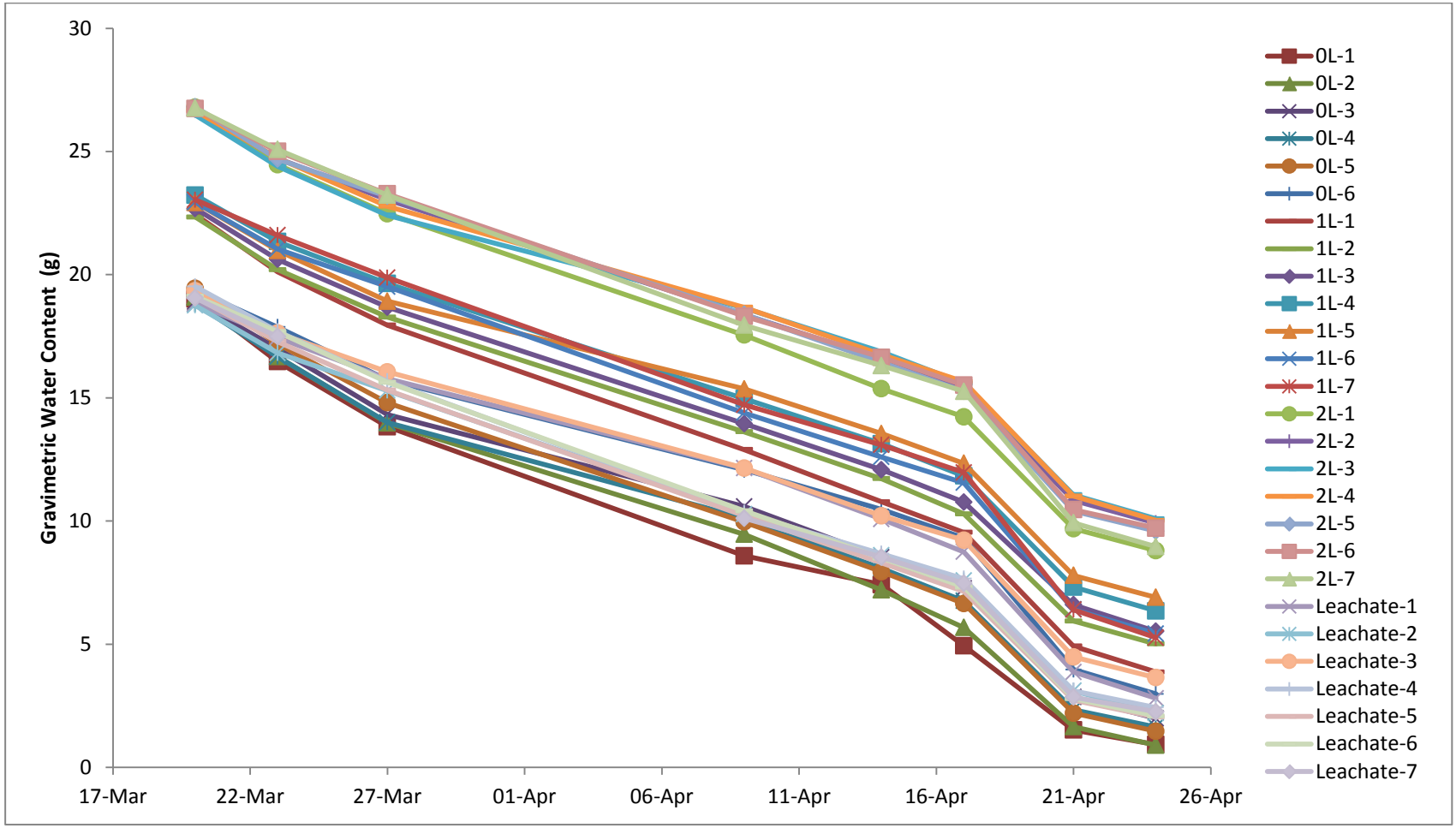

Figure C Gravimetric soil water content for individual incubation jars with varying amounts of litter, where 0L is no litter, 1L is single litter and 2L is double litter addition. .