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Effects of drying–rewetting frequency on soil carbon and nitrogen transformations

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Abstract

Soil drying and rewetting impose a significant stress on the soil microbial community. While wetting events are common in most environments, the short and long-term effects of soil rewetting on microbial processes have not been well studied. Furthermore, it is not clear if stress history is important to consider when modeling microbial controls on ecosystem dynamics. In this experiment, we manipulated the frequency of soil rewetting events during 2 months to determine how stress history influences the response of soil microbial communities to rewetting events. Two soils were collected from the Sedgwick Ranch Natural Reserve in Santa Ynez, CA, one from an annual grassland, the other from underneath an oak canopy. Soils were incubated in the lab and went through either 0, 1, 2, 4, 6, 9, or 15 drying–rewetting cycles over 2 months. Soil moisture content was adjusted so that the average moisture content over the course of the incubation was the same for all samples, compensating for the number of drying–rewetting cycles. Soils were analyzed for respiration rate, substrate utilization efficiency, nitrification potential, microbial biomass, and NH_4^+ and NO_3^- concentrations. Total CO_2 loss during incubation significantly increased with number of rewetting events for oak soils but not for grass soils, where a large number of rewetting events decreased total CO_2 loss. Exposure to frequent drying–rewetting events decreased the amount of CO_2 released upon rewetting and dramatically increased the activity of autotrophic nitrifier populations. For up to 6 weeks after the last drying–rewetting cycle, respiration rates in soils exposed to a history of drying–rewetting events were substantially lower than their non-stressed controls. In all cases, the effects of the rewetting stress were greater in oak than in grass soils. The results indicate that drying–rewetting events can induce significant changes in microbial C and N dynamics and these effects can last for more than a month after the last stress. The frequency of drying–rewetting stress events has important ecosystem-level ramifications and should be incorporated into models of soil microbial dynamics.

Keywords: Drying–rewetting; Stress; Respiration; Nitrifiers; Soil carbon

1. Introduction

In most terrestrial ecosystems, surface soils experience periods of drying followed by relatively rapid rewetting. Soils of Central California, and other semi-arid, Mediterranean-type ecosystems, are particularly susceptible to drying–rewetting stresses due to the infrequency of rainfall events and the often warm, dry climate that favors rapid soil drying. Understanding the effects of these drying–rewetting events on soil microbial processes is important to our understanding of ecosystem C and N dynamics in these systems.

Both C and N mineralization rates generally increase for a few days following the rewetting of a dry soil (Birch, 1958; Bloem et al., 1992; Cui and Caldwell, 1997; Franzluebbers et al., 2000). The source of this 'pulse' of C and N is unclear. The rapid change in soil water potential associated with rewetting may cause microbes to undergo osmotic shock, inducing microbial cell lysis (Bottner, 1985; Van Gestel et al., 1992) or a release of intracellular solutes (Halverson et al., 2000). These labile C and N substrates could then be rapidly mineralized by the remaining microbes, yielding a pulse of C and N (Birch, 1959; Kieft et al., 1987). Alternatively, drying–rewetting may cause soil aggregates to break apart, exposing physically protected organic matter (Adu and Oades, 1978; Lundquist et al., 1999a). This previously unavailable organic matter could then be rapidly mineralized by the microbial community (Appel, 1998).

While we do know that drying–rewetting yields a 1–4 d increase in C and N mineralization rates, we know little about the longer term implications of drying–rewetting stresses for ecosystem C and N dynamics. After the short-term pulse of C and N, do soil processes return to the same pre-stress equilibrium state? If so, it is relatively

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unimportant to incorporate drying–rewetting dynamics into soil process models because the C and N pulse is of relatively short duration (Cabrera, 1993) and of limited magnitude in annual C and N transformations. However, if a drying–rewetting event causes the soil to assume a new equilibrium for C and N transformation rates (relative to an unstressed soil), or if the recovery to pre-stress basal rates is slow, then the incorporation of drying–rewetting events into models that adequately predict ecosystem C and N fluxes becomes more important and more difficult. Studies conducted by Clein and Schimel (1994) and Bottner (1985) suggest that this may be the case with microbial processes affected for extended periods after a drying–rewetting event.

In the field, most soils experience, not one, but a series of drying–rewetting events throughout the course of the year. Few studies have examined how the frequency of these stress events (the rewetting stress history) controls soil processes. Schimel et al. (1999) showed that the frequency of drying–rewetting events altered microbial biomass and respiration on decomposing birch litter. Likewise, a field study by Fay et al. (2000) suggests that the length of time between rainfall events controls soil CO_2 flux. On the other hand, the majority of ecosystem models assume a roughly linear response function (below field capacity) between soil water availability and C and N mineralization rates (Rodrigo et al., 1997). These models are driven by moisture in the current time step and do not include moisture history. Thus, a linear response to moisture produces an implicit result that the average moisture over time should predict microbial activity as well as integrating the individual values at different times, regardless of the wetting and drying history. In this vein, most larger-scale biogeochemical models do assume that any variability in soil moisture content can be integrated temporally and the average moisture values can be used to adequately predict C and N mineralization rates (McGill, 1996; Parton et al., 1987). We designed this experiment partly to test these model assumptions which will increase in importance in the future with global climate change scenarios predicting a change in the seasonal pattern of rainfall events (Easterling, 1990; Houghton et al., 1990). If the frequency of drying–rewetting events controls soil processes, then the variability in rainfall during a given period, not just the average rainfall, would have to be incorporated into models of soil C and N dynamics.

The specific questions addressed by this experiment include the following. What are the short-term (1–3 d) and long-term (1 month) effects of soil drying–rewetting on C and N dynamics? Does drying–rewetting stress history, when isolated from changes in average water content, influence soil processes? Are these responses to drying–rewetting events similar between different soil types? Can longer term (months) microbial dynamics be predicted simply from knowing the average water content with time, or does the variation in moisture need to be taken into account?

2. Methods

2.1. Soils

The two soils used for this experiment were collected from the University of California Sedgwick Reserve, a 2364 ha reserve located in Santa Ynez, California, USA (34°42′30″N, 120°2′30″W). The climate is Mediterranean, with relatively wet winters and very dry, hot summers. The soils of the field site are Haploxerolls (Gessler et al., 2000). Surface soils (0–10 cm) were collected from underneath perennial oak (*Quercus agrifolia*) and from an adjacent annual grassland (primarily *Bromus* spp.). These soils will be referred to as 'oak' and 'grass' soil, respectively. These two soils were chosen because they are found in close proximity to one another, yet are distinct with respect to composition and local microclimate. The oak soil had a higher total C and N content (3.9 and 0.3%, respectively) than the grass soils (2 and 0.2%, respectively), more available N, higher nitrification rates, and a larger microbial biomass pool (Fierer, unpublished data). The oak soil is a loam with a pH in water of 6, the grass soil is a clay loam with a pH of 6.5. Seasonally the oak soil would subjected to fewer rapid changes in soil water content than the grass soil due to a thicker litter layer and canopy shading.

2.2. Experimental setup

Prior to the start of the experiment, the soils were sieved to 4 mm, homogenized, and conditioned for 1 week at 35% of water holding capacity (WHC) (37 and 28% gravimetric water content for oak and grass soils, respectively). 100% WHC was measured as the gravimetric water content of soil saturated and allowed to drain over 6 h in a filter funnel. Treatments were done in triplicate with 10–15 g of soil incubated at 20 °C in sealed glass 2 l Mason jars with weights measured periodically to assure the correct soil moisture content. For the experiment, soils were incubated for 2 months and exposed to six different drying–rewetting stress regimes: either 1, 2, 4, 6, 9, or 15 drying–rewetting events during the course of the experiment (Fig. 1). The control treatment consisted of soils kept at a constant moisture content, 35% WHC, which corresponds to a water potential of approximately −60 kPa in both soils, as measured on a thermocouple psychrometer (Decagon Devices, Inc. Model SC-10a). The drying–rewetting events were evenly spaced throughout the 2 month incubation with all of the treatments receiving the final (or only in the case of the soils that received only one) drying–rewetting event at the end of the incubation. Drying–rewetting events consisted of a 2 d drying period followed by a rewetting. Soil drying was accomplished by removing the jar lids and incubating in a 20 °C room with substantial air flow. By the end of the drying period, the soils always dried down close to 5% gravimetric water content (approximately −15 MPa for both soils). Soils were rewetted by adding a single

aliquot of a pre-determined amount of deionized water to the middle of the soil sample.

Each of the stressed samples had the water content after rewetting adjusted to achieve an average water content of 35% WHC during the 2 month incubation. To maintain all the treatments at the same average water content, the frequently dried and rewet soils were adjusted to a higher post-rewetting water content. Preliminary studies were conducted to determine soil drying curves during the 'drying period' and these data were used to calculate the average water contents. For example, the oak soils that received six drying–rewetting events during the course of the incubation were adjusted to 42% gravimetric water content while the oak soils that received 15 drying–rewetting events were adjusted to 49% (Fig. 1). An unstressed 50% WHC treatment (53 and 40% gravimetric water content for oak and grass soils, respectively) was included to account for the higher adjusted moisture contents of the soils that received numerous drying–rewetting stresses; this treatment was wetter than any of the cycling soils. Both oak and grass soils had water potentials of approximately −40 kPa at 50% WHC. Overall, this experimental setup allowed us to manipulate the frequency of drying–rewetting events without altering average soil water content over the 2 month incubation.

In this experiment, all soils were adjusted to the same average water content over the course of the experiment, not the same average water potential, which is more likely to control microbial activity (Harris, 1981). The experiment was designed in this manner for two reasons. First, most models of soil organic matter dynamics use water content or a related moisture index, not water potential, as a control on microbial activity (Rodrigo et al., 1997) and the experiment was designed to test the assumptions of these models. Second, maintaining all the soils at the same average water potential over the incubation would be methodologically challenging since water potential is difficult to manipulate accurately, especially with the hysteresis associated with frequent drying–rewetting events (Hillel, 1980).

2.3. Respiration measurements

During the course of the incubation (and post-incubation periods), respiration rates were determined by measuring CO_2 concentrations in each jar at regular intervals. Jar headspace was sampled through rubber septa on Mason jar lids with a glass syringe and CO_2 was analyzed on a gas chromatograph (Shimadzu model 14 with a 2 m Porapak Q column running at 45 °C with a thermal conductivity detector). The jars were vented after each sampling to keep headspace CO_2 concentrations from exceeding 2%. Respiration rates were monitored continuously in the samples that received drying–rewetting events to account for any increased flux of CO_2, released by rewetting. Respiration rates during the drying portion of the incubation were measured on a subset of samples using an infrared gas analyzer (Licor Model LI-6252) pumping at a speed of 40 ml min^{-1}. A flowthrough lid was placed over each jar for 5–15 min, depending on the degree of drying, after which, the lid was removed and the samples returned to drying. Average respiration, rates over the course of the incubation were calculated by summing the CO_2 produced during each 'moist' sampling interval, and, if applicable, by integrating the CO_2 measurements taken during the drying portions of the incubation.

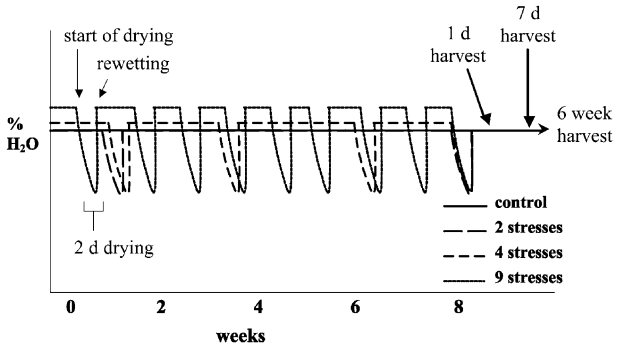

Fig. 1. A diagram representing the experimental setup (not to scale) with a few of the treatments and harvests used as examples.

2.4. Post-incubation analyses

At the end of the 2 month incubation, all samples were adjusted to a constant moisture content (35% WHC). Samples were then incubated for an additional 6 weeks. Respiration rates were monitored during this time. Soil was harvested from both the control treatments and the treatments receiving 1, 2, 6, and 15 drying–rewetting events for nitrification potential and substrate utilization assays at 1 and 7 d after the last drying–rewetting event. Microbial biomass and extractable nutrients were measured on harvested soil samples from all treatments at 1, 7, and 40 d after the final stress event.

Nitrification potential, performed using the short-term chlorate slurry inhibition assay (Belser and Mays, 1980), was used as an indirect estimate of autotrophic nitrifier biomass. Approximately 5 g of soil was used per assay and NO_2^- concentrations in the slurry were measured every 2 h during a 6 h incubation.

Extractable NH_4^+ and NO_3^- concentrations were determined by 1 h extraction with 0.5 M K_2SO_4 and subsequent analysis of the filtered extract on a Lachat autoanalyzer. Ammonium was analyzed using the diffusion method (Lachat method 31-107-06-5-A, Milwaukee, WI) and nitrate was analyzed using Griess-Ilovsay reaction after Cd reduction (Lachat method 12-107-04-1-B, Milwaukee, WI).

Microbial biomass C and N were measured using the chloroform fumigation–extraction technique (Vance et al., 1987). Extracts were analyzed for total C and total N using a persulfate digestion technique (A.P. Doyle, unpublished).

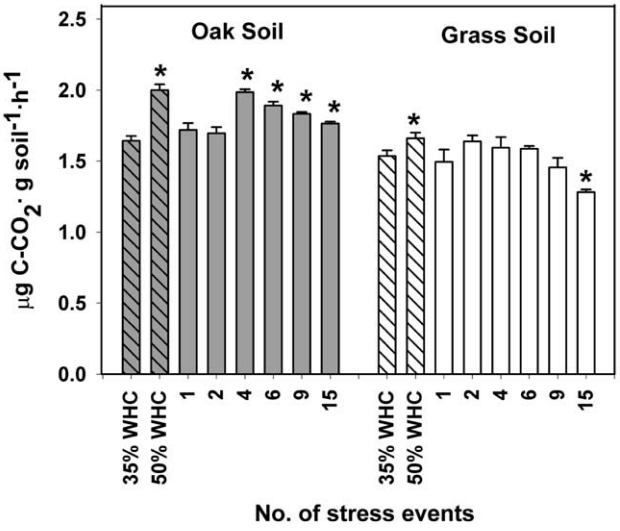

Fig. 2. Average respiration rate during the 2 month incubation when drying–rewetting frequencies were manipulated. The 35 and 50% WHC unstressed controls are indicated by hatched bars. * = significantly different ($P < 0.05$) from the 35% WHC control.

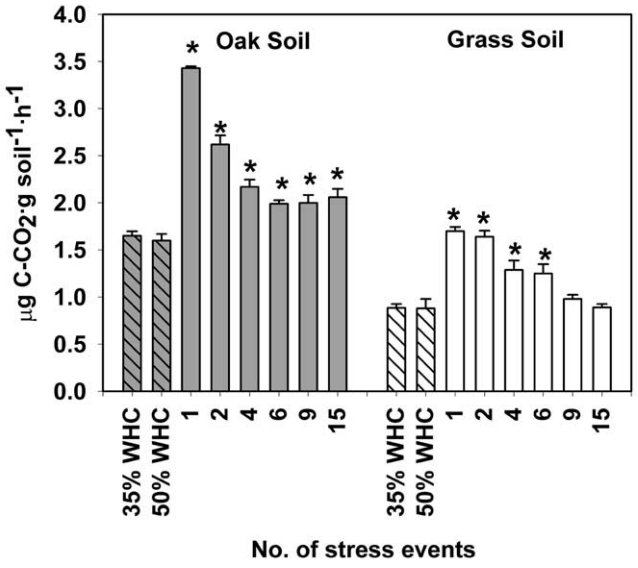

Fig. 3. Respiration rates during the first 24 h after the last drying-rewetting. For the stressed treatments, this is an index of the size of the rewetting CO_2 pulse. The 35 and 50% WHC unstressed controls are indicated by hatched bars. * = significantly different ($P < 0.05$) from the 35% WHC control.

Microbial biomass C and N content was calculated by subtracting the total C and N in unfumigated samples from that in fumigated samples. Biomass C and N values were *not* corrected for extraction efficiency and thus represent a 'flush' of C and N instead of the total microbial biomass.

Substrate utilization was assayed with glucose and glutamic acid added as substrates (Sugai and Schimel, 1993). Citric acid and salicylic acid were also used but their recovery efficiencies were prohibitively low (15 and 20%, respectively) so these data will not be reported. The technique involved adding a 50 μl aliquot of universally labeled ^{14}C substrate (1 μCi ml^{-1}) to a pair of 5 g soil samples. The soils were incubated in a gas-tight 50 ml tube with phenylethylamine CO_2 traps for 24 h at 20°C. After incubation, the CO_2 traps were removed and one of the samples was extracted immediately by shaking for 1 h with a 0.5 M K_2SO_4 solution, while the other was fumigated for 24 h with chloroform and then extracted in the same manner. Both the fumigated and unfumigated extracts and the CO_2 traps were mixed with Universol and counted on a liquid scintillation counter. This technique allowed us to determine the proportions of added substrate incorporated into biomass, respired as CO_2, or left unconsumed in the soil.

2.5. Statistical analyses

One way ANOVAs were used for each soil type to compare treatments (Systat 10 for Windows 2000). If there was a significant treatment effect ($P < 0.05$), Fisher's LSD tests were used to determine which differences between treatments were significant. Two different unstressed control treatments were used in this study. Since all samples were adjusted to the same average water content (35% WHC), the 35% WHC controls were compared to the stressed treatments to isolate the effects of stress frequency at a common average water content, on C and N dynamics. The 50% WHC controls were compared to the 35% WHC controls to determine if adjusting the frequently stressed soils to higher water contents upon rewetting had any effects on C and N transformations.

3. Results

3.1. Soil respiration

During the 2 month incubation, the average flux of CO_2 from oak soils that received four or more stress events was higher than the control ($P < 0.01$ in all cases) (Fig. 2). The greatest increase was 24% for the four-stress treatment. The average respiration rate for the oak 50% WHC control was 22% higher than that for the oak 35% WHC control ($P < 0.001$).

The grass soils showed a different response to the frequency of stress events (Fig. 2). While average respiration rates were not significantly affected by a low number of stress events, the grass soils exposed to 15 stress events had significantly lower average respiration rates than the control ($P = 0.007$). During the 2 month incubation, the 50% WHC control had 10% higher average respiration compared to the 35% WHC control ($P = 0.04$).

Respiration rates were measured for 24 h following the final rewetting event to estimate the size of the last rewetting CO_2 pulse (Fig. 3). All the treatments (except the control) were subjected to this final drying–rewetting event and since all soils were maintained at 35% WHC after this

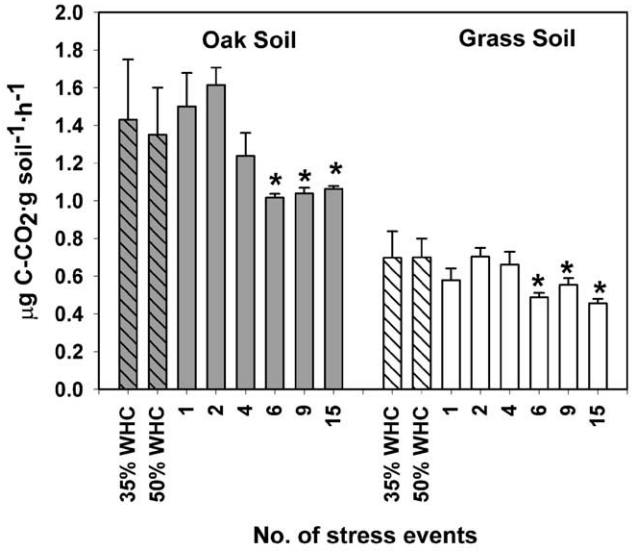

Fig. 4. Soil respiration rates 6 weeks after the last drying–rewetting. The 35 and 50% WHC unstressed controls are indicated by hatched bars. * = significantly different ($P < 0.05$) from the 35% WHC control.

stress, the magnitude of the rewetting stress was the same for all treatments. All the stressed oak soils had respiration rates significantly higher than the control ($P < 0.003$ in all cases). After the final rewetting, only the grass soils exposed to six or fewer previous stresses had higher respiration rates than the control ($P < 0.001$). For the single stress treatment, both the oak and grass soils had rewetting pulses of approximately equal magnitude, with respiration rates being approximately twice the control. In both soils, the magnitude of the rewetting CO_2 pulse was inversely proportional to the number of drying–rewetting events the soil had experienced during the 2 month incubation.

Respiration rates were monitored for 6 weeks after the final drying–rewetting event. The oak soils that received 6, 9, and 15 drying–rewetting events had approximately 35% lower respiration rates than the control ($P < 0.03$ in all cases) 3 weeks (data not shown) and 6 weeks (Fig. 4) after the end of the experimental treatments. The frequently stressed grass samples (6, 9, and 15 stresses) had respiration rates that were approximately 25% lower than the control at both 3 weeks and 6 weeks ($P < 0.05$ in all cases). In both soils, the respiration rates of the 50% WHC unstressed treatments were statistically indistinguishable from those of the 35% WHC controls 3 and 6 weeks after the incubation.

3.2. Extractable nitrogen and carbon

Soil extractable NH_4^+ concentrations in both soil types were very low and were not affected by the stress treatments. On average, NH_4^+ accounted for less than 5% of total extractable inorganic N.

In oak and grass soils, there were no statistically significant effects of stress treatment on soil extractable NO_3^- concentrations either 1 or 7 d after the final drying–rewetting event (data not shown). However, 6 weeks after the final stress event there was a significant decrease in extractable NO_3^- concentrations in both the oak and grass soils that were stressed repeatedly (Fig. 5). In both soils, the decrease in soil extractable NO_3^- was most substantial when soils were exposed to numerous drying–rewetting cycles. The 50% WHC controls from both soils had very similar NO_3^- concentrations to the 35% WHC controls when extracted at 6 weeks following stress termination ($P = 0.2$ for oak, $P = 0.6$ for grass).

In both soils, dissolved organic C (DOC) concentrations showed no significant differences or obvious trends between treatments on unfumigated extractions conducted 1 and 7 d after the last stress (data not shown). However, in oak soils extracted 6 weeks after the last stress, there was a significant decrease in DOC concentrations when comparing frequently stressed soils to the control (Fig. 6). This effect was also evident in the grass soils but the decrease in DOC concentrations was only evident in the soils stressed 9 and 15 times ($P < 0.03$ in both cases). At 6 weeks, the oak soil had slightly lower DOC concentrations (approximately 6%) in the 50% WHC control compared to the 35% WHC control ($P = 0.002$), but the two grass controls were almost identical ($P = 0.5$).

Dissolved organic N (DON) concentrations are not reported due to the very high variability between soil extracts. Since DON concentrations were typically a small fraction (<6%) of extractable NO_3^- concentrations, any variability in the persulfate digestion technique introduced a significant amount of variability to estimates of DON concentrations.

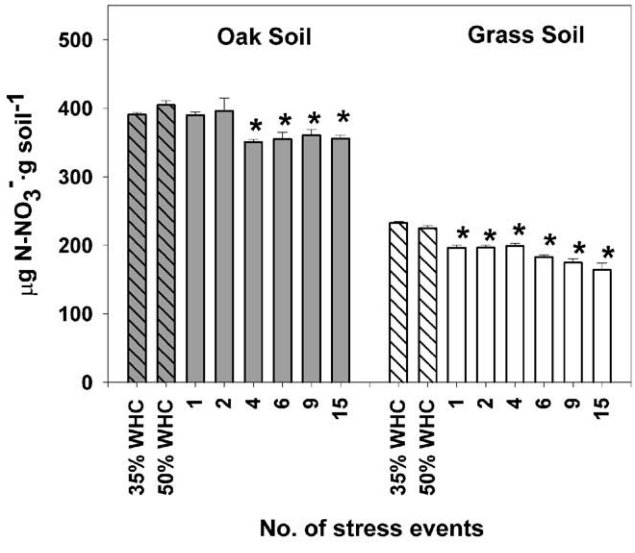

Fig. 5. Soil extractable NO_3^- concentrations, 6 weeks after the last drying–rewetting The 35 and 50% WHC unstressed controls are indicated by hatched bars. * = significantly different ($P < 0.05$) from the 35% WHC control.

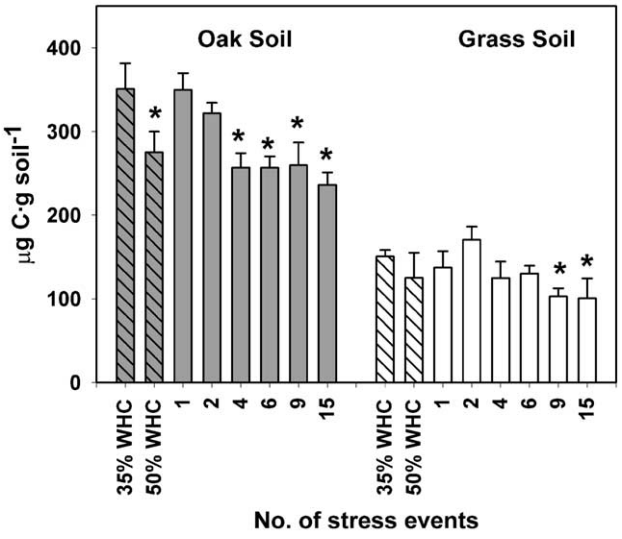

Fig. 6. Soil extractable organic carbon (K_2SO_4-extractable) 6 weeks after the last drying–rewetting. The 35 and 50% WHC unstressed controls are indicated by hatched bars. * = significantly different ($P < 0.05$) from the 35% WHC control.

3.3. Microbial biomass

The size of the soil microbial C pool was not immediately affected by the stress treatments. The estimates of biomass C obtained from fumigation extractions made 1 and 7 d after the final drying–rewetting event yielded no significant differences in the biomass C concentrations of either soil when comparing the controls to the stress treatments (data not shown). At the 6-week time point, the frequently stressed oak soils had significantly higher biomass C than the control (Fig. 7). The grass soils also showed a trend towards increasing biomass C with the number of stress cycles ($r^2 = 0.48$, $P = 0.09$) (Fig. 7). The 2 oak unstressed controls had similar levels of biomass C ($P = 0.4$), but the grass 50% WHC control had significantly higher biomass C than the grass 35% WHC control ($P = 0.02$). Data regarding the microbial N pool are not reported due to the difficulty in measuring organic N concentrations (see earlier).

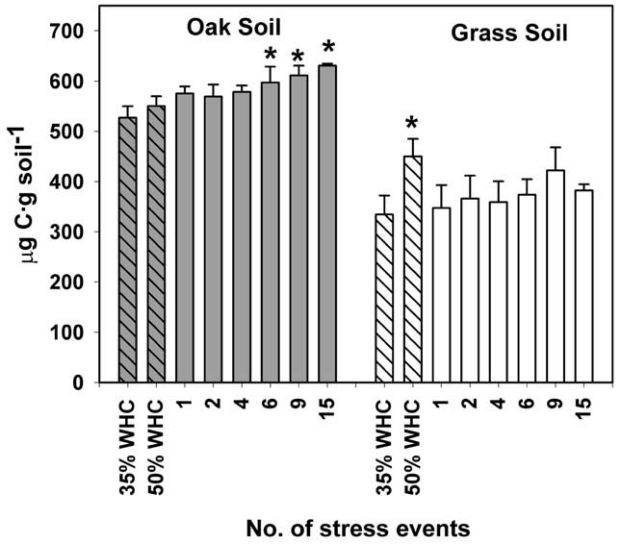

Fig. 7. Soil flush C (fumigation-extractable biomass C) 6 weeks after the last drying–rewetting. The 35 and 50% WHC unstressed controls are indicated by hatched bars. * = significantly different ($P < 0.05$) from the 35% WHC control.

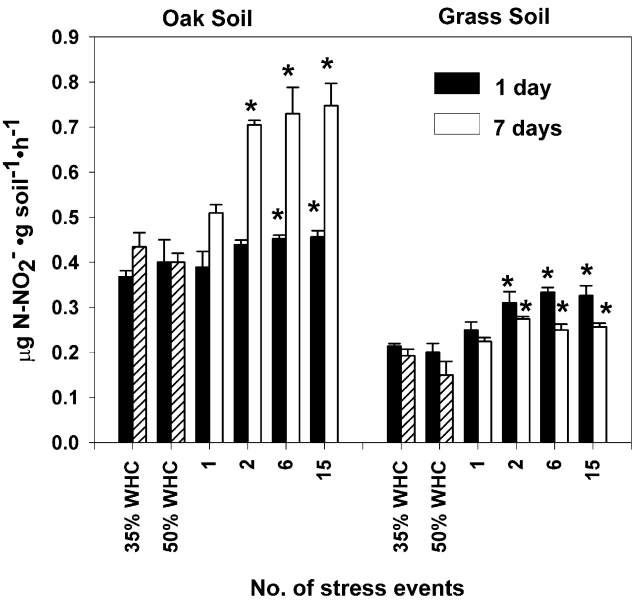

Fig. 8. Soil nitrification potentials (an index of autotrophic nitrifier biomass) 1 and 7 d after the last drying–rewetting. The 35 and 50% WHC unstressed controls are indicated by hatched bars. * = significantly different ($P < 0.05$) from the 35% WHC control.

3.4. Nitrification potentials

Nitrification potentials increased with frequent drying and rewetting (Fig. 8). In oak soils, there was a significant increase in nitrification potentials in the frequently stressed treatments compared to the control. This increase was very considerable 7 d after the last rewetting. Frequent drying–rewetting events also significantly increased nitrification potentials in grass soils but the increase was less than in the oak soils (Fig. 8). In both soil types, the 50% WHC control had nitrification potentials almost identical to the 35% WHC controls at both time points ($P > 0.2$ in all cases).

3.5. Substrate use efficiencies

Substrate use efficiencies are reported as the ratio of the added substrate ^{14}C in the flush (fumigation extractable biomass) over that given off as CO_2. A higher ratio indicates a higher use efficiency. Grass soil exhibited no significant trends between the use efficiencies of glucose or glutamic acid and the frequency of drying–rewetting events (Fig. 9). In oak soil, the efficiencies of glucose use significantly decreased 1 d after the last stress in stressed soils versus the control ($P < 0.05$ in all cases), i.e. the microbial

communities exposed to stress incorporated less of the added glucose into biomass relative to the amount respired. This decrease in glucose use efficiencies was no longer evident by at 7 d (Fig. 9). The glutamic acid use efficiencies by oak soils showed different trends. There was a minimal treatment effect 1 d after the last stress, but after 7 d, the glutamic acid use efficiencies in the stress treatments increased relative to the control (Fig. 9). Glucose and glutamic acid use efficiencies in the oak 50% WHC control were approximately 8% lower than the 35% WHC control at both time points but these differences were not significant.

The effects of stress treatments on substrate utilization efficiencies were not due to differences in the total amount of ^{14}C recovered, which remained relatively constant for each substrate and soil type (data not shown). In both soils, approximately 50% of the added ^{14}C-glucose and 65% of the added ^{14}C-glutamic acid was accounted for in the K_2SO_4 extracts or as CO_2, the remaining ^{14}C was probably bound to clays or in non-extractable microbial biomass and thus not recovered.

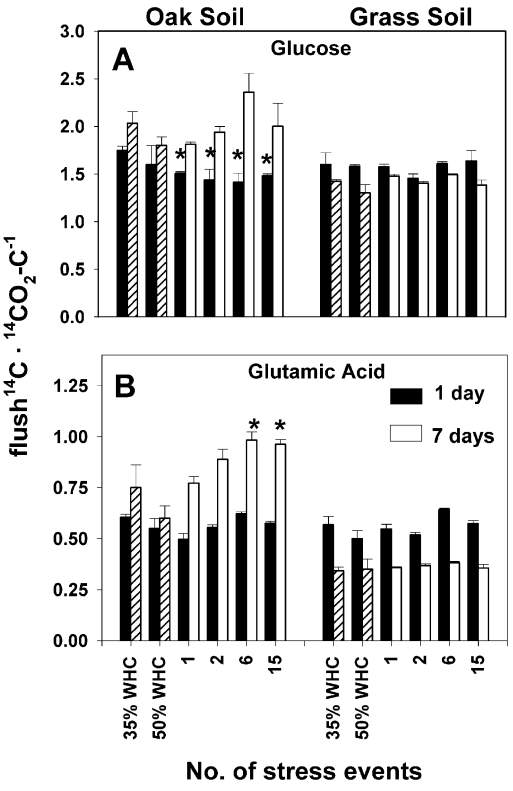

Fig. 9. Substrate use efficiencies (ratio of ^{14}C substrate incorporated into biomass to that released as CO_2) for glucose (A) and glutamic acid (B), 1 and 7 d after the last drying–rewetting. The 35 and 50% WHC unstressed controls are indicated by hatched bars. * = significantly different ($P < 0.05$) from the 35% WHC control.

4. Discussion

4.1. C dynamics

The frequency of drying–rewetting events has particularly clear ecosystem consequences for soil C mineralization rates. Many field and laboratory studies have shown that soil respiration rates are strongly influenced by average water content (Howard and Howard, 1993). We have shown that variability in water content alone, with no change in average water content, can have both short term (up to 1 week) and longer term (up to 6 weeks) effects on the rates of soil C mineralization.

4.1.1. Rewetting CO_2 pulse

Fig. 3 shows that stress history plays an important role in determining the magnitude of the rewetting CO_2 pulse. In both soils, the more rewetting events previously experienced, the less CO_2 released after a rapid rewetting. Since this pattern cannot be explained by reductions in the size of the microbial biomass pool, there are two other possible explanations. One, if drying–rewetting releases physically protected soil organic matter, there may be simply less organic matter available for release following a series of drying–rewetting events, reducing the size of the CO_2 pulse. Two, after a few drying–rewetting events, the microbial community may adjust to the water potential shock encountered during rewetting (Van Gestel et al., 1993; Lundquist et al., 1999b). This adjustment would lessen the mortality rate and reduce the size of the flush of labile substrate available for mineralization by surviving microbes. The community change in response to rewetting stress could result from a selection for a group of microbes, such as fungi or Gram positive bacteria, that can withstand the osmotic shock (Harris, 1981) and the incapacitation of intolerant microbes in the community. Adjustment could also occur with no change in community structure if the pre-existing microbes develop thicker polysaccharide protective layers (Roberson and Firestone, 1992) or accumulate greater concentrations of osmoprotectant solutes (Kempf and Bremer, 1998) to cope with rapid water potential fluctuations. Our inability to definitively identify the cause of the rewetting pulse makes it difficult to explain this relationship between stress history and the size of the rewetting CO_2 pulse.

4.1.2. C dynamics during the 2 month incubation

Over the 2 month incubation, the soils that received multiple drying–rewetting events had average respiration rates that were either 10% higher (oak soil) or 10% lower (grass soil) than their controls. These small changes in average respiration rates are particularly striking considering that the soils that received multiple stresses spent a substantial amount of the time (up to 30 d in the most frequently stressed soils) drying during this 2 month incubation. The pulse of CO_2 released after each rewetting event must have been large enough to either overcompensate, in the case of the oak soil, or almost compensate, in the case of the grass

soil, for the reduced respiration rates during the drying periods.

Why did frequently stressed oak soils have higher respiration rates than the control over the course of the incubation while grass soils showed the opposite pattern? Relative to the respective controls, the rewetting pulses during the 24 h period were the same for both oak and grass soils (Fig. 3), but over a 3-day period, oak soils respired 30% more than grass soils, relative to the 35% WHC control (Fierer, unpublished data). Therefore, as a proportion of the unstressed controls, oak soils had a larger pulse of CO_2 following a rewetting compared to the grass soils. As mentioned, this C respired after rewetting may come from either biomass turnover (Kieft et al., 1987) or the release of previously unavailable soil organic matter (Adu and Oades, 1978). The oak soil had both a larger biomass pool (Fig. 7) and a larger total pool of soil organic matter, so either mechanism could explain the larger rewetting pulse in oak soils.

Overall, the increased variability in moisture content can increase or decrease average respiration rates, by, at most 10–15%, depending on soil type. These results are very similar to those obtained by exposing tundra and taiga soils to freeze thaw stresses (Schimel and Clein, 1996) suggesting that the two types of stress have similar effects on soil processes. A 10–15% deviation from the control would be small compared to the degree of error inherent in models that attempt to predict soil respiration rates from monthly or weekly average water contents. If one were to look solely at the 2 months when drying–rewetting frequencies were altered, one would likely conclude that it would not be particularly important to incorporate the degree of variability around an average water content into a predictive model of soil respiration rates.

4.1.3. Stress history and long-term recovery of C dynamics

If we look at the longer-term effects of drying–rewetting stress history on soil respiration rates we see significantly greater responses compared to the short-term effects. Our results agree with those of Magid et al. (1999), Franzluebbers et al. (1994), and Schimel et al. (1999) who found that drying–rewetting can retard long-term C mineralization rates. Both 3 and 6 weeks (Fig. 4) after the last stress, the soils that received multiple stresses had substantially lower respiration rates than the controls. This reduction in respiration rates was due solely to the effects of stress history, since the control and treated soils were incubated at the same temperature and moisture content for the 6 weeks following the last stress.

The frequently dried and rewetted soils were adjusted to higher moisture contents during each rewetting so they would have the same average water content as the 35% WHC control. One could argue that the decrease in long-term respiration rates was not a product of the frequent drying–rewetting stresses per se, but of the higher moisture content to which the frequently stressed soils were adjusted. However, because the 50% WHC unstressed treatments had comparable respiration rates to the 35% WHC controls 3 and 6 weeks after the last stress (Fig. 4), this argument is not valid.

Frequent drying–rewetting would undoubtedly reduce soil structure and water holding capacities (Adu and Oades, 1978), increasing water potentials at a given water content and reducing soil aeration (Hillel, 1980). Because our wetter controls, at 50% WHC, had respiration rates similar to the 35% WHC controls after the 2 month incubation, it is not likely that any breakdown in soil structure could explain the longer term effects of drying–rewetting frequency on C mineralization rates. Any increase in water potential or decrease in aeration would be of greater magnitude in the 50% WHC controls than in the wettest treatments.

A decrease in the supply of remaining mineralizable organic matter following a period of frequent rewettings would be the most parsimonious explanation for the reduction in respiration rates. Presumably, after a single rewetting the pool of potentially mineralizable soil organic matter would increase due to either a release of physically protected organic matter or a 'priming effect' caused by the release of labile substrates during biomass turnover. Thus, with time, a series of rewetting events could serve to reduce the total supply of available organic matter. The measured dissolved organic C contents, which may serve as an index of mineralizable organic matter supply (Boyer and Groffman, 1996), support this hypothesis. Six weeks after the last stress, the frequently stressed oak and grass soils had a decrease in DOC contents of approximately 30% relative to the controls. These decreases in DOC contents correspond to the 35 and 28% decreases in respiration rates for frequently stressed oak and grass soils, respectively.

However, if frequent rewetting events served to lower organic matter supply, one would expect a corresponding increase in either biomass C or respiration rates over the course of the 2 month incubation. In both soils, biomass C concentrations were the same as the control immediately after the last stress (data not shown), only after 6 weeks, and only in the oak soils, did we see a treatment effect on biomass C. In addition, during the 2 month incubation the frequently stressed oak soils respired only 10% more than the control and the grass soils respired 10% less than the control. The observed changes in biomass C at 6 weeks and the respiration rates during the 2 month incubation may explain a portion of the 35% decrease in respiration rates for the oak soils, but does not explain the observed 28% decrease in grass soil respiration rates, which were lower during the 2 month incubation. A change in the supply of available organic matter cannot completely explain the decrease in long-term respiration rates observed in the frequently stressed soils.

4.1.4. Microbial community and C dynamics?

Another potential explanation for the reduction in long-term respiration rates is a change in the composition of the

microbial community. Not all members of the microbial community are equally adept at mineralizing SOM (Waldrop et al., 2000). Loss of some of these microbes by drying–rewetting, or a change in the physiologies of the extant population (Insam, 1990) could reduce the ability of the microbial community to catabolize soil organic matter, as has been demonstrated by Schimel et al. (1999). The only direct evidence we have for a change in community functioning comes from the substrate use data. We observed that substrate use efficiencies were altered by stress history in oak soils, but not in grass soils, 1 and 7 d after the last stress (Fig. 9). Unfortunately, substrate use data were not collected at 3 or 6 weeks. The data from this assay must be interpreted with a degree of caution since the changes were inconsistent between 1 and 7 d and the assay only measures the utilization of simple substrates (glucose or glutamic acid), not the utilization efficiencies of native pools of soil C which are much more complex and difficult to break down.

We know that drying–rewetting events can alter microbial community structure (Lundquist et al., 1999b; McLean and Huhta, 2000); so a change in community composition in response to a history of drying–rewetting events is a distinct possibility. Analyses of bacterial community structure from this same experiment (N.G. Fierer, P.A. Holden, J.P. Schimel, unpublished data) show that the bacterial community composition did change in response to drying–rewetting frequency, particularly in oak soils. The changes in community composition could result in a change in microbial functioning and a subsequent decrease in long-term respiration rates.

4.2. Stress frequency and microbial biomass

The pool of microbial biomass C was increased by frequent exposure to drying–rewetting events in the oak soil (Fig. 7). There were no short-term effects of stress history on biomass C, the effects were only evident 6 weeks after the last stress, once the soils seemed to have approached an equilibrium state. This finding runs contrary to a number of studies which have shown a decrease in microbial biomass after exposure to drying–rewetting events (Sorensen, 1983; Van Gestel et al., 1996). Compared to microbes from soils that rarely experience extreme fluctuations in moisture content, the microbes found in soils from a Mediterranean climate may be better adapted to frequent drying–rewetting events.

An increase in organic matter availability following a rewetting may lead to a period of rapid microbial growth (Lund and Goksoyr, 1980). These microbes could then persist in a semi-dormant state for an extended period even after the flush of resources has been exhausted, resulting in the observed net long-term biomass accumulation in frequently stressed soils.

4.3. N dynamics and stress history

The frequency of drying–rewetting events also has specific ramifications for soil N transformations. The nitrification potential assays suggest a substantial increase in the autotrophic nitrifier population in frequently stressed oak and grass soils (Fig. 8). This increase was not due to the higher water content to which frequently stressed soils were rewetted, the 50% WHC unstressed samples had nitrification potentials almost identical to the control (35% WHC). This observed increase in nitrification potentials was surprising considering that nitrifiers are generally considered to be highly sensitive to moisture stress (Stark and Firestone, 1995) and Franzluebbers et al. (1994) observed a decrease in nitrification rates with repeated drying–rewetting events. We propose that while nitrifier activity may generally be sensitive to periods of low moisture, the nitrifiers found in the oak and grass soils were able to survive the drying periods. Low nitrifier mortality during the drying periods coupled with the ability of nitrifiers to thrive on a flush of NH_4^+ released during rewetting (Birch, 1959; Cabrera, 1993), may lead to an overall increase in nitrifier biomass and activity in frequently dried and rewet soils.

An increase in nitrifier biomass should correspond to an increase in nitrification rates in stressed soils. However, 6 weeks after the last stress, soil NO_3^- concentrations were actually lowered in soils with a history of multiple drying–rewetting events. The enhanced uptake of NO_3^- by the microbial biomass may have exceeded any enhanced NO_3^- production. While NO_3^- is not the favored form of N for microbial anabolism, when NH_4^+ concentrations are low, as they are in these soils, NO_3^- consumption can be substantial (Davidson et al., 1990). The increase in microbial biomass in frequently stressed soils may explain the increase in NO_3^- consumption. Assuming a biomass C to N ratio of 5:1, in both soils the increase in biomass C must be on the order of 200 μg C g soil^{-1} to account for the 40 μg N g soil^{-1} reductions in NO_3^- concentrations. If fumigation extraction efficiencies are approximately 30% (Ross, 1990) and the microbial biomass uses NO_3^- as the sole N source, the total increases in biomass for frequently stressed soils (300 and 200 μg biomass C g soil^{-1} for oak and grass soils, respectively) would be sufficient to explain the observed decreases in NO_3^- concentrations after 6 weeks. NO_3^- concentrations may have also been lowered in the frequently stressed soils due to pulses of denitrification following rewetting. Other studies have shown a short-term increase in NO and N_2O emissions following a drying–rewetting event (Groffman and Tiedje, 1988; Davidson et al., 1993; Scholes et al., 1997); however, it is not clear if denitrification rates would have been of sufficient magnitude to explain the reductions in NO_3^- concentrations.

4.4. Ecosystem level implications

This experiment implies that exposing soils from this

ecosystem type to multiple drying–rewetting events can result in moderate short-term increases (or decreases, depending on soil type) in respiration rates, substantial reductions in long-term respiration rates, an increase in nitrifier activity, and an increase in the size of the microbial biomass C pool.

Fluctuations in soil moisture content could substantially increase losses of N from soils in this ecosystem type. The enhanced rates of nitrification may increase leaching losses of N as NO_3^- and gaseous losses of N, via nitrification or denitrification.

The influence of stress history on C mineralization in these soils is still detectable 6 weeks after the last stress. After numerous stresses, the soils do not return to the same equilibrium respiration rates as the unstressed soils. Long-term rates of soil C mineralization may be substantially lowered by a high degree of variability in soil moisture. To accurately model microbial controls on soil C and N cycling, at least in soils, such as these, from Mediterranean ecosystems, the variability in soil water content, as well as the average water content, must be considered.

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References

Adu, J., Oades, J., 1978. Physical factors influencing decomposition of organic materials in soil aggregates. Soil Biology & Biochemistry 10, 109–115.

Appel, T., 1998. Non-biomass soil organic N: the substrate for N mineralization flushes following soil drying–rewetting and for organic N rendered $CaCl_2$-extractable upon soil drying. Soil Biology & Biochemistry 30, 1445–1456.

Belser, L.W., Mays, E.L., 1980. Specific inhibition of nitrate oxidisation by chlorate and its use in assessing nitrification in soils and sediments. Applied and Environmental Microbiology 39, 505–510.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9–31.

Birch, H.F., 1959. Further observations on humus decomposition and nitrification. Plant and Soil 9, 262–286.

Bloem, J., Ruiter, P.D., Koopman, G., Lebbink, G., Brussaard, L., 1992. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. Soil Biology & Biochemistry 24, 655–665.

Bottner, P., 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ^{14}C and ^{15}N labelled plant material. Soil Biology & Biochemistry 17, 329–337.

Boyer, J.N., Groffman, P.M., 1996. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. Soil Biology & Biochemistry 28, 783–790.

Cabrera, M., 1993. Modeling the flush of nitrogen mineralization caused by drying and rewetting soils. Soil Science Society of America Journal 57, 63–66.

Clein, J., Schimel, J., 1994. Reduction in microbial activity in birch litter due to drying and rewetting events. Soil Biology & Biochemistry 26, 403–406.

Cui, M., Caldwell, M., 1997. A large ephemeral release of nitrogen upon wetting of dry soil and corresponding root responses in the field. Plant and Soil 191, 291–299.

Davidson, E., Stark, J., Firestone, M., 1990. Microbial production and consumption of nitrate in an annual grassland. Ecology 71, 1968–1975.

Davidson, E., Matson, P., Vitousek, P., Riley, R., Dunkin, K., Garcia-Mendez, G., Maass, J., 1993. Processes regulating soil emissions of NO and N_2O in a seasonally dry tropical forest. Ecology 74, 130–139.

Easterling, W., 1990. Climate trends and prospects. In: Sampson, R., Hair, D. (Eds.). Natural Resources for the 21st Century. Island Press, Washington, DC, pp. 32–55.

Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M., Collins, S.L., 2000. Altering rainfall timing and quantity in a mesic grassland ecosystem: design and performance of rainfall manipulation shelters. Ecosystems 3, 308–319.

Franzluebbers, K., Weaver, R.W., Juo, S.R., Franzluebbers, A.J., 1994. Carbon and nitrogen mineralization from cowpea plants part decomposing in moist and in repeatedly dried and wetted soil. Soil Biology & Biochemistry 26, 1379–1387.

Franzluebbers, A., Haney, R., Honeycutt, C., Schomberg, H., Hons, F., 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Science Society of America Journal 64, 613–623.

Gessler, P.E., Chadwick, O.A., Chamran, F., Althouse, L., Holmes, K., 2000. Modeling soil-landscape and ecosystem properties using terrain attributes. Soil Science Society of America Journal 64, 2046–2056.

Groffman, P., Tiedje, J., 1988. Denitrification hysteresis during wetting and drying cycles in soil. Soil Science Society of America Journal 52, 1626–1629.

Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64, 1630–1637.

Harris, R., 1981. Effect of water potential on microbial growth and activity. In: Parr, J., Gardner, W., Elliott, L. (Eds.). Water Potential Relations in Soil Microbiology. Soil Science Society of America, Madison, pp. 23–95.

Hillel, D., 1980. Fundamentals of Soil Physics. Academic Press, New York.

Houghton, J., Jenkins, G., Ephraums, J. (1990). Climate Change. The IPCC Scientific Assessment. World Meteorological Organization. Cambridge University Press, Cambridge.

Howard, D., Howard, P., 1993. Relationships between CO_2 evolution, moisture content, and temperature for a range of soil types. Soil Biology & Biochemistry 25, 1537–1546.

Insam, H., 1990. Are the soil microbial biomass and basal respiration governed by the climatic regime? Soil Biology & Biochemistry 22, 525–532.

Kempf, B., Bremer, E., 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high osmolality environments. Archiv fur Mikrobiologie 170, 319–330.

Kieft, T., Soroker, E., Firestone, M., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biology & Biochemistry 19, 119–126.

Lund, V., Goksoyr, J., 1980. Effects of water fluctuations on microbial mass and activity in soil. Microbial Ecology 6, 115–123.

Lundquist, E., Jackson, L., Scow, K., 1999a. Wet dry cycles affect DOC in two California agricultural soils. Soil Biology & Biochemistry 31, 1031–1038.

Lundquist, E., Scow, K., Jackson, L., Uesugi, S., Johnson, C., 1999b. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. Soil Biology & Biochemistry 31, 1661–1675.

Magid, J., Kjaergaard, C., Gorissen, A., Kuikman, P., 1999. Drying and rewetting of a loamy sand soil did not increase the turnover of native

organic matter, but retarded the decomposition of added ^{14}C labelled plant material. Soil Biology & Biochemistry 31, 595–602.

McGill, W.B., 1996. Review and classification of ten soil organic matter (SOM) models. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.). Evaluation of Soil Organic Matter Models. Springer, Berlin, pp. 113–132.

Mclean, M.A., Huhta, V., 2000. Temporal and spatial fluctuations in moisture affect humus microfungal community structure in microcosms. Biology and Fertility of Soils 32, 114–119.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173–1179.

Roberson, E., Firestone, M., 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Applied and Environmental Microbiology 58, 1284–1291.

Rodrigo, A., Recous, S., Neel, C., Mary, B., 1997. Modelling temperature and moisture effects on C–N transformations in soils: comparisons of nine models. Ecological Modelling 102, 325–339.

Ross, D., 1990. Estimation of soil microbial C by a fumigation–extraction method: influence of seasons, soils, and calibration with the fumigation-incubation procedure. Soil Biology & Biochemistry 22, 295–300.

Schimel, J., Clein, J., 1996. Microbial response to freeze–thaw cycles in tundra and taiga soils. Soil Biology & Biochemistry 28, 1061–1066.

Schimel, J.P., Gulledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology & Biochemistry 31, 831–838.

Scholes, M., Martin, R., Scholes, R., Parsons, D., Winstead, E., 1997. NO and N_2O emissions from savanna soils following the first simulated rains of the season. Nutrient Cycling in Agroecosystems 48, 115–122.

Sorensen, L., 1983. The influence of stress treatments on the microbial biomass and the rate of decomposition of humified matter in soils containing different amounts of clay. Plant and Soil 75, 107–119.

Stark, J., Firestone, M., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218–221.

Sugai, S., Schimel, J., 1993. Decomposition and biomass incorporation of ^{14}C labeled glucose and phenolics in taiga forest floor: effect of substrate quality successional state, and season. Soil Biology & Biochemistry 25, 1379–1389.

Van Gestel, M., Ladd, J., Amato, M., 1992. Microbial biomass responses to seasonal change and imposed drying regimes at increasing depths of undisturbed topsoil profiles. Soil Biology & Biochemistry 24, 103–111.

Van Gestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast- and slow growing microorganisms in soils from different climates. Soil Biology & Biochemistry 25, 109–123.

Van Gestel, M., Merckx, R., Vlassak, K., 1996. Spatial distribution of microbial biomass in microaggregates of a silty-loam soil and the relation with the resistance of microorganisms to soil drying. Soil Biology & Biochemistry 28, 503–510.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial C. Soil Biology & Biochemistry 19, 703–707.

Waldrop, M.P., Balser, T.C., Firestone, M.K., 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32, 1837–1846.