DIVISION S-3—SOIL BIOLOGY & BIOCHEMISTRY

A Proposed Mechanism for the Pulse in Carbon Dioxide Production Commonly Observed Following the Rapid Rewetting of a Dry Soil

Noah Fierer* and Joshua P. Schimel

ABSTRACT

The rapid rewetting of a dry soil often yields a pulse in soil CO_2 production that persists for 2 to 6 d. This phenomenon is a common occurrence in surface soils, yet the mechanism responsible for producing the CO_2 pulse has not been positively identified. We studied the effects of a single drying and rewetting event on soil C pools, to identify which specific C substrates are mineralized to produce the observed pulse in respiration rates. We labeled two soils with ^{14}C-glucose and measured the enrichment and pool sizes of the released CO_2, extractable biomass C, and extractable soil organic matter (SOM-C) throughout a drying and rewetting cycle. After rewetting, respiration rates were 475 to 370% higher than the rates measured before the dry down. The enrichment of the released CO_2 was 1 to 2 times higher than the enrichment of the extractable biomass C pools and 10 to 20 times higher than the enrichment of the extractable organic C, suggesting that the CO_2 pulse was generated entirely from the mineralization of microbial biomass C. However, there was no evidence of substantial microbial cell lysis on rewetting. We hypothesize that the pulse of CO_2 is generated by the rapid mineralization of highly enriched intracellular compounds as a response by the microbial biomass to the rapid increase in soil water potentials. The drying and rewetting process also releases physically protected SOM, increasing the amount of extractable SOM-C by up to 200%. The additional SOM-C rendered soluble by the rewetting event did not contribute substantially to the rewetting CO_2 pulse. Overall, the rapid rewetting of a dry soil can influence soil C cycling in the short-term, by increasing the microbial mineralization of cytoplasmic solutes, and in the longer-term, by decreasing the total amount of SOM physically protected within microaggregates.

Numerous studies have shown that the rapid rewetting of a dry soil can cause a large pulse in soil C mineralization rates (Birch, 1958; Clein and Schimel, 1994; Franzluebbers et al., 2000; Jager and Bruins, 1975; Soulides and Allison, 1961). After a soil rewetting, CO_2 production rates are often elevated by as much as 500% compared with samples kept continuously moist, with the CO_2 pulse generally persisting for a 2- to 6-d period following the rewetting event. Since many surface soils experience large seasonal fluctuations in moisture content, these short-term pulses in CO_2 production after rewetting are likely to be a common occurrence in many soils. In arid, semi-arid, or Mediterranean environments, where rainfall events are infrequent and soils are often dry, the rewetting CO_2 pulse may constitute a significant proportion of the total annual CO_2 flux from surface soils.

There is uncertainty about the mechanisms responsible for producing the rewetting CO_2 pulse. One proposed mechanism is that the pulse of CO_2 is largely a result of the mineralization of nonbiomass soil organic matter (SOM) rendered accessible to microbial attack by the rewetting event. According to this hypothesis, the drying and rewetting process disrupts aggregate structure, releasing organic matter from physical protection within aggregates and producing a pulse in microbial activity as this material is mineralized (Adu and Oades, 1978; Appel, 1998; Denef et al., 2001; Sorensen, 1974; Utomo and Dexter, 1982). Alternatively, others have proposed that microbial C, not SOM-C, is the major substrate mineralized to produce the rewetting CO_2 pulse (Bottner, 1985; Kieft et al., 1987). The rapid increase in soil water potential associated with the rewetting of a dry soil causes microbes to experience osmotic shock. In general, microbial cells either lyse completely or adjust to the water potential shock by releasing intracellular osmoregulatory solutes (Halverson et al., 2000; Harris, 1981). The compounds released into the soil environment are taken up by surviving microbes and mineralized, producing the respiration pulse. Some studies have combined these two proposed mechanisms, suggesting that both biomass C and nonbiomass SOM-C contribute to the rewetting CO_2 pulse (Scheu and Parkinson, 1994; Van Gestel et al., 1993a; Van Gestel et al., 1991; Van Veen et al., 1985).

The identification of the specific mechanisms responsible for producing the rewetting CO_2 pulse is important if we want to understand the implications of climate change on soil C dynamics. In the future, many regions of the globe may experience higher mean annual temperatures and greater intra-annual variability in the timing of precipitation events (Barrow and Hulme, 1996; Houghton et al., 1996; Waggoner, 1989). Under these scenarios, we would expect many surface soils to experience more frequent drying and rewetting events. If nonbiomass SOM-C is the primary source of the rewetting CO_2 pulse, an increase in the frequency of soil drying and rewetting will increase the amount of soil C accessible to microbial attack, potentially decreasing the total amount of C sequestered in a particular soil over time. However, if microbial biomass is the source of the rewetting CO_2 pulse, an increase in the frequency of drying-rewetting events may increase the level of physiological stress for soil microbes, potentially reducing C mineralization and increasing C sequestration rates over time.

We conducted an experiment with two soils from a

N. Fierer and J. P. Schimel, Dep. of Ecology, Evolution, and Marine Biology, Univ. of California, Santa Barbara, CA 93106. Received 1 July 2002. *Corresponding author (fierer@lifesci.ucsb.edu).

Published in Soil Sci. Soc. Am. J. 67:798–805 (2003).

Abbreviations: SOM, soil organic matter.

Mediterranean-type environment to determine if biomass or nonbiomass SOM is the substrate mineralized to produce the rewetting CO_2 pulse and to identify the specific mechanisms involved in the process. We labeled the microbial biomass pool with ^{14}C glucose and measured the enrichment of the CO_2 after rewetting to assess the relative contributions of labeled microbial biomass C and unlabeled SOM-C to the CO_2 pulse. We propose a conceptual model of rewetting C dynamics that explains the results from this experiment as well as the results obtained from similar studies.

MATERIALS AND METHODS

The two soils used for the experiment were collected from the University of California Sedgwick Reserve in Santa Ynez, CA (N 34°42′30″ lat., W 120°2′30″ long.). The climate is Mediterranean, with an average annual rainfall of 50 cm yr^{-1} (Cachuma Reservoir, Santa Barbara County Water District) and most of the rainfall occurring between the months of November and March. The soils of the field site are Haploxerolls (Gessler et al., 2000). Surface soils (0–10cm) were collected from underneath perennial oak (*Quercus agrifolia*) and from adjacent annual grassland (primarily *Bromus* spp.). These soils will be referred to as "oak" and "grass" soils, respectively. The oak soil has a higher total C and N content (3.9 and 0.3%, respectively) than the grass soil (2 and 0.2%, respectively), more available N, higher nitrification rates, and a larger microbial biomass (Fierer and Schimel, 2002). 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 be subjected to fewer rapid fluctuations in soil water content than the grass soil because of a thicker litter layer and canopy shading (Parker and Muller, 1982).

The soil samples were collected in April 2001, sieved to 4 mm, and stored at field moistures (22 and 12% for oak and grass soils, respectively) at 5°C. Three weeks before the start of the experiment (August 2001) the two soils were adjusted to field capacity (−0.03 MPa), as measured on a thermocouple psychrometer (Decagon Devices, Inc. Pullman, WA; Model SC-10a). Field capacity corresponds to gravimetric H_2O contents of 55 and 42% for the oak and grass soils, respectively.

A schematic diagram of the experimental setup is provided in Fig. 1. Four replicate samples of each soil type were weighed into individual 50-mL centrifuge tubes, each sample weighing 7 g (dry weight equivalent). All samples were kept at 20°C throughout the course of the experiment. A 1-mL aliquot of universally labeled ^{14}C-glucose (28 kBq mL^{-1}, 3.4 nmol glucose mL^{-1}) was added to each sample, raising the water potential of the oak and grass soil samples from −0.03 MPa to approximately −0.01 MPa. After an 8-d pre-incubation, we started monitoring total CO_2 and $^{14}CO_2$ production rates from the samples by placing NaOH traps (2 mL of 0.2 *M* NaOH) inside each gas-tight centrifuge tube. Sodium hydroxide traps inside empty tubes were used to determine the background levels of CO_2 and ^{14}C-CO_2. Traps were removed on a regular basis and stored in a CO_2–free environment until analyzed.

Twenty days after the ^{14}C glucose addition, all samples were dried over a 3-d period and then rewet back to the pre-dry down water content. The relatively long incubation period allowed for the equilibration of the ^{14}C into the microbial biomass C pools before dry-down. Soil water potentials were maintained at a constant level (−0.01 MPa) for the entire period after the addition of the ^{14}C glucose and before drying (Fig. 1). Soil drying was accomplished by placing samples directly beneath a low powered fan in a 20°C room. By the end of the drying period, both oak and grass soil samples had dried down close to −30 MPa (approximately 5% gravimetric water content). All soil samples were rewetted back to −0.01 MPa by adding a single aliquot of a pre-determined amount of deionized water to the middle of the sample. Soils were never removed from their respective tubes, physically agitated, or heated during the course of the drying and rewetting process.

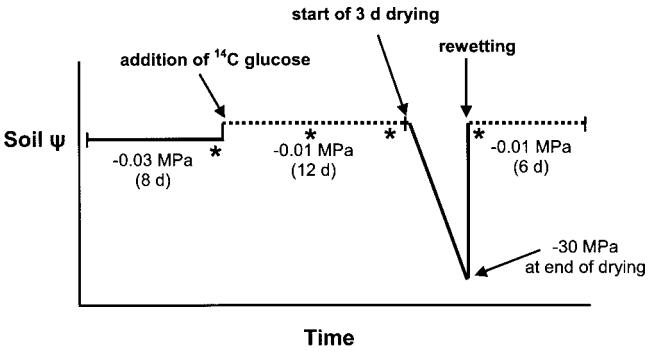

Fig. 1. A schematic diagram (not to scale) illustrating the time course of events and the soil water potentials during the experiment. Oak and grass soil samples were adjusted to identical soil water potentials. * equals a set of samples were destructively harvested for unfumigated and fumigated extractions. Dashed lines indicate the time periods during which we monitored CO_2 and ^{14}C-CO_2 production.

Immediately after the rewetting, we continued monitoring total CO_2 and $^{14}CO_2$ production rates for 6 d, switching NaOH traps every 12 or 24 h. The NaOH traps were analyzed for total dissolved C with a flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). After converting dissolved bicarbonate to CO_2 using HCl, CO_2 was measured by running the sample over a Teflon membrane with phenol red as a color indicator (a modification of the Lachat ammonium diffusion method, 31-107-06-5-A, Lachat Instruments Milwaukee, WI). Headspace ^{14}C-CO_2 accumulation was measured by counting an aliquot of each NaOH trap on a liquid scintillation counter. Both ^{14}C-CO_2 and CO_2 flux rates were calculated for each sample after subtracting the amounts of ^{14}C and CO_2 in the respective "blank" NaOH traps.

An additional set of soil samples were treated in exactly the same manner as those described above, but were destructively harvested during the course of the experiment for unfumigated and fumigated extractions. The harvests were conducted at three time points: 6 d before drying, 1 d before drying, and immediately following the rewetting (Fig. 1). We conducted a preliminary harvest after the 8-d pre-incubation period and before the ^{14}C labeling to determine if the addition of the glucose led to a substantial increase in the amount of biomass C. At each harvest, four replicate soil samples were extracted with and without $CHCl_3$ using a method modified from Gregorich et al. (1990). Briefly, for unfumigated and fumigated extractions, samples were transferred to 70-mL glass tubes (Pyrex No. 9825) and 40 mL of 0.5 *M* K_2SO_4 was added to each sample. For fumigated extractions, 0.5 mL of EtOH-free $CHCl_3$ was added directly to the sample along with the K_2SO_4 solution. Blanks, extract solution without soil, were prepared both with and without the addition of $CHCl_3$. All extracts were shaken for 3 h at 150 rpm and gravity filtered through Whatman No. 1 paper (Whatman, Maidstone, UK). The extracts were then bubbled vigorously with air for 30 min to remove any $CHCl_3$ and stored at −20°C. In terms of biomass extraction efficiencies, this method is directly comparable with vapor-phase chloroform fumigation techniques (N. Fierer, unpublished data, 2001).

Total dissolved organic C concentrations in soil extracts were measured by persulfate digestion (Cabrera and Beare, 1993) with the inorganic C concentrations of the digested samples analyzed using the method described above. Dissolved C concentrations in the K_2SO_4 blanks with and without $CHCl_3$ addition were subtracted from the fumigated and unfumigated extract concentrations, respectively. Extractable organic C levels were determined from the unfumigated K_2SO_4 extractions. Aliquots of both unfumigated and fumigated extracts were analyzed on a liquid scintillation counter to measure the quantity of ^{14}C in extractable DOC and biomass C. Microbial biomass C and ^{14}C flush values are reported as the difference in extracted C, or ^{14}C, between fumigated and unfumigated samples, without conversion to total microbial biomass C. Statistical analyses of the extract data were conducted using one way ANOVAs with Fisher's LSD post-hoc tests (SPSS, Inc., 2000).

RESULTS

After exposure to a drying and rewetting event, a CO_2 pulse was evident in both the oak and grass soils (Fig. 2). After the rewetting, respiration rates were elevated by as much as 475 and 370% (oak and grass soils, respectively) relative to the respiration rates measured before drying. Approximately 5 to 6 d after rewetting, respiration rates in both soils returned to the pre-dry down levels. Over the entire 6-d period following the rewetting, an average of 71 and 36 μg $C\text{-}CO_2$ g $soil^{-1}$ were released from the oak and grass soils, respectively. By calculating the amount of $C\text{-}CO_2$ released during an equivalent 6-d period before drying (46 and 29 μg $C\text{-}CO_2$ g $soil^{-1}$, oak and grass) we can estimate, by difference, that the rewetting CO_2 pulse itself was approximately 25 and 7 μg $C\text{-}CO_2$ g $soil^{-1}$ for oak and grass soils, respectively.

Before drying, the rates of $^{14}C\text{-}CO_2$ release (Fig. 3a) and the enrichments of the CO_2 (Fig. 3b) were relatively constant for both soils. Following rewetting, we observed an increase in the rates of $^{14}C\text{-}CO_2$ release from both soil types (Fig. 3a). This pulse of $^{14}C\text{-}CO_2$ release is of relatively short duration, lasting for approximately

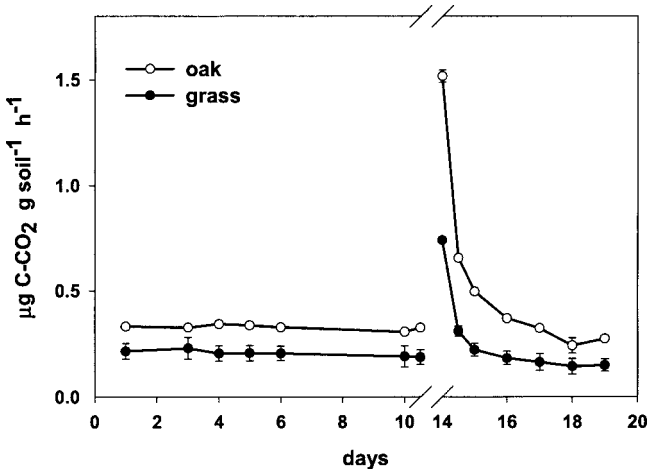

Fig. 2. Rates of total CO_2 production before and after rewetting. Carbon dioxide production was not monitored during the drying period, Day 11 to Day 13. Error bars represent ±1 standard error of the means (N = 4).

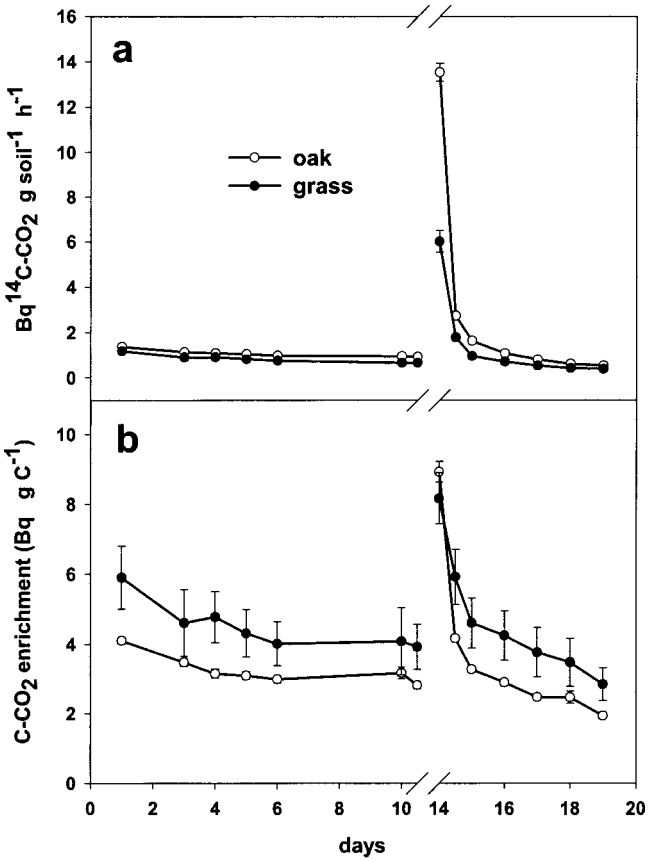

Fig. 3. Carbon-14 labeled CO_2 release rates (2a) and the enrichment of respired CO_2 (2b) before and after rewetting. Error bars represent ±1 standard error of the means (N = 4).

1 to 3 d before rates return to the basal rates observed before drying. For both soil types, the relative size of the $^{14}C\text{-}CO_2$ pulse on rewetting was greater in magnitude than the pulse in total CO_2 production, producing an overall increase in CO_2 enrichment after rewetting (Fig. 3b). Carbon dioxide enrichments immediately following the rewetting were 2 to 3 times higher than the CO_2 enrichments measured before the drying.

The addition of the ^{14}C-glucose (0.48 nmol [34.6 ng] glucose-C g $soil^{-1}$) did not lead to any measurable net microbial growth in either soil type. Extractable biomass C levels before glucose addition, 225 (SE = 20) and 140 (SE = 14) μg C g $soil^{-1}$ for oak and grass soils respectively, were nearly equivalent to the levels of biomass C extracted 14 d after glucose addition (6 d before drying, Fig. 4a).

The pool sizes of extractable microbial biomass C in both oak and grass soils were not substantially affected by rewetting. The amounts of extractable microbial biomass C were not significantly different between samples collected 1 d before drying and samples collected immediately after rewetting (Fig. 4a). Similarly, the ^{14}C enrichments of the microbial biomass pools from both soils were not significantly different in samples extracted just before drying or just after rewetting (Fig. 4b). The enrichment of the extractable biomass C, as measured following rewetting, was approximately 3.4 and 4.8 Bq g $soil^{-1}$ for oak and grass soils, well below the measured

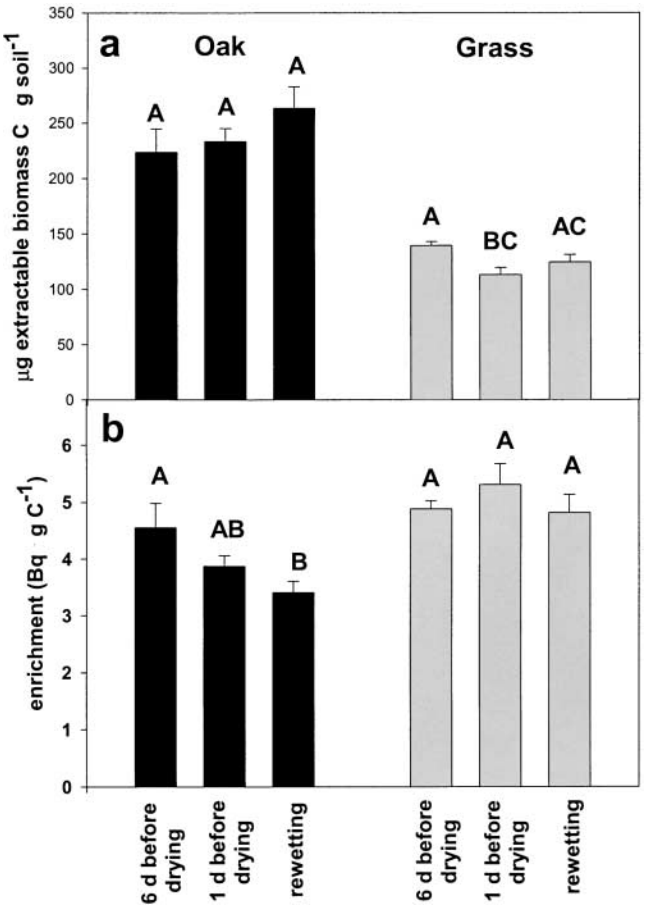

Fig. 4. The amount (3a) and enrichment (3b) of extractable microbial biomass C recovered from oak and grass soils at three time points during the experiment. Error bars represent 1 standard error of the means ($N = 4$). Means denoted by the same letter do not differ significantly ($P > 0.05$).

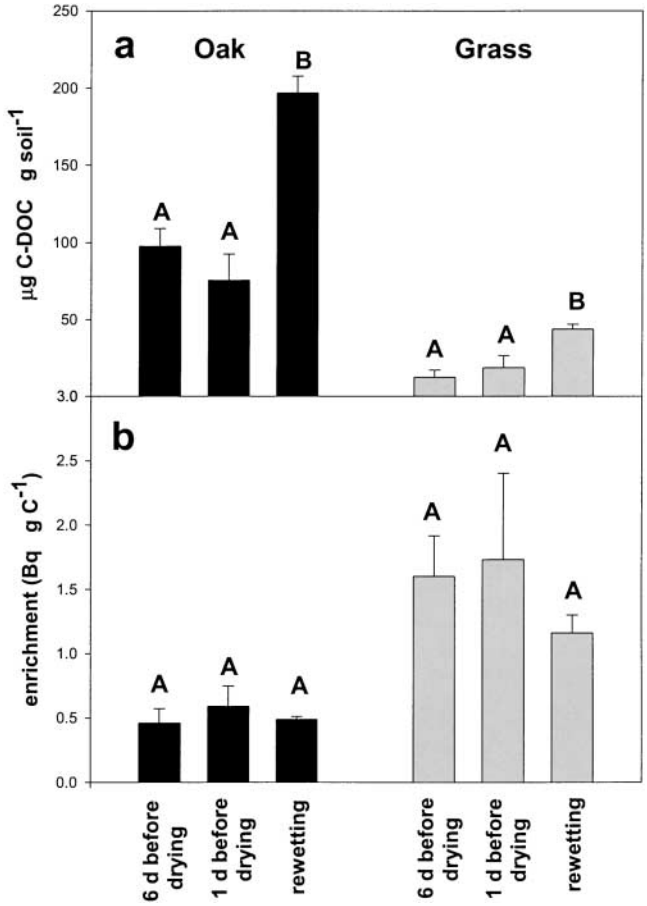

Fig. 5. The total amount (4a) of extractable-organic C from an unfumigated extraction with 0.5 M K_2SO_4 and the enrichment of the extracted C (4b). Error bars represent 1 standard error of the means ($N = 4$). Means denoted by the same letter do not differ significantly ($P < 0.05$).

levels of enrichment for the CO_2 released soon after rewetting.

In both soils, the pool sizes of extractable organic C were significantly increased by the drying and rewetting process (Fig. 5a). Soils extracted immediately after rewetting had over 200% more organic C than soils extracted before the drying. These increases in extractable C concentrations were not accompanied by a change in the enrichment of the C (Fig. 5b). With both soil types, there were no significant differences in the levels of enrichment of the extractable organic C at the three different extraction times. The enrichments of extractable C were generally 4 to 10 times lower than the enrichment of biomass C in both soil types.

DISCUSSION

The Source of the Rewetting CO_2 Pulse

The high ^{14}C enrichment of the CO_2 released after rewetting is evidence that microbial C, not SOM-C, is the primary substrate mineralized after a soil-rewetting event. If SOM-C was a significant source of the CO_2 pulse, we would expect the enrichment of the rewetting pulse CO_2 to be lower than the CO_2 released before drying since SOM-C is largely unlabeled. If the microbial C was uniformly labeled and was the sole source of C mineralized to produce the rewetting CO_2 pulse, we would expect the enrichment of the rewetting pulse CO_2 to be nearly equivalent to the enrichment of the microbial biomass C. Interestingly, with both soil types the enrichment of the CO_2 released immediately after rewetting was nearly double the enrichment of the extractable microbial biomass C.

There are two possible explanations why the rewetting-pulse CO_2 is more ^{14}C enriched than the extractable microbial biomass pool. One explanation, the "active biomass" hypothesis, is that the added ^{14}C is preferentially incorporated into metabolically active microbes which are sensitive to being lysed on rewetting and the mineralization of the lysed cells produces a pulse of highly enriched CO_2. Another possible explanation is that a subfraction of the microbial biomass is more highly enriched with ^{14}C than the overall microbial biomass and this highly enriched fraction is mineralized on rewetting. Although we are not able to identify the specific composition of the highly enriched cellular material, it is likely to consist of soluble cytoplasmic material, hence we term this hypothesis the "cytoplasmic solute" hypothesis.

The active biomass hypothesis has been used to ex-

plain similar results from other drying and rewetting experiments with ^{14}C-labeled soil. Bottner (1985) and Van Gestel et al. (1993b) proposed that actively growing microbes are preferentially enriched in ^{14}C and have thinner cell walls, making them more susceptible to cell lysis on rewetting than slower growing microbes. The subsequent mineralization of the lysed active biomass would then produce a CO_2 pulse with a higher level of ^{14}C enrichment than the total extractable biomass C pool. Although we cannot refute this hypothesis with the data available from this study, the active biomass hypothesis is not likely to be the most parsimonious explanation for our results. We added a much smaller amount of C (36 ng C g soil^{-1}) than in the experiments by Bottner (1985) and Van Gestel et al. (1993b) and these low concentrations of glucose should not be sufficient to cause a net increase in the active microbial biomass pool. Furthermore, we have no evidence that the rewetting event induced cell lysis: there was no measurable reduction in the size of the microbial biomass pool after rewetting (if anything, microbial biomass tended to increase, Fig. 4a) and the ^{14}C enrichments of the unfumigated extracts collected immediately after rewetting are low (Fig. 5b). The technique we used to measure microbial biomass may not have had the sensitivity to measure the relatively small changes in biomass needed to account for the rewetting CO_2 pulse (5–10% of microbial biomass). However, we should have been able to detect an increase in the ^{14}C enrichments of the unfumigated extracts if even a small proportion of the microbial biomass had lysed in response to the rewetting.

Our data suggest, but do not necessarily confirm, that the cytoplasmic solute hypothesis is a more plausible explanation for the high enrichment of the rewetting pulse CO_2. Cytoplasmic C should have a higher level of ^{14}C enrichment than the overall extractable biomass C pool because any added ^{14}C is more likely to be incorporated into cytoplasmic constituents than structural components, particularly if low concentrations of ^{14}C-labeled substrate are added to soil (Bremer and Van Kessel, 1990; Bremer and Kuikman, 1994). We speculate that the large pulse of highly enriched CO_2 released after a drying and rewetting cycle is a result of the mineralization of highly enriched cytoplasmic solutes by cells responding to the water potential shock. The rewetting-induced mineralization of cytoplasmic C, with limited cell lysis, may explain why a number of studies have observed an increase in respiration rates after the rewetting of a dried soil and no significant reduction in microbial numbers (Bloem et al., 1992; Fierer and Schimel, 2002; Lundquist et al., 1999a; Magid et al., 1999; Scheu and Parkinson, 1994).

This proposed explanation for the rewetting CO_2 pulse is supported by a number of pure-culture studies examining the physiological responses of microbes to dilution stress. At low water potentials, bacteria and fungi accumulate cytoplasmic solutes, primarily low molecular weight carbohydrates, polyols, amino acids, and amines, to lower intracellular water potentials and maintain cell turgor (Csonka and Hanson, 1991; Harris, 1981; Potts, 1994). A rapid increase in water potentials (a dilution stress) can induce microbial cell lysis unless intracellular water potentials are immediately raised by the release of these cytoplasmic solutes into the surrounding environment (Halverson et al., 2000; Sleator and Hill, 2001). After release, the cytoplasmic solutes are rapidly (in as little as 10 min.) re-incorporated into the microbial cells (Hohmann, 2002; Ruffert et al., 1997; Schleyer et al., 1993; Tschicholz and Truper, 1990; Wood et al., 2001) where they are eventually mineralized or re-assimilated (Csonka and Hanson, 1991; Dylan et al., 1990; Reed and Stewart, 1983; Wood et al., 2001).

We hypothesize that the microbes in the oak and grass soils rapidly mineralize cytoplasmic solutes on rewetting, producing a pulse of highly enriched CO_2. These solutes could possibly be microbial osmoregulants that accumulate intracellularly during low water potential conditions and are subsequently removed by mineralization or assimilation on rewetting of the soil. If this hypothesis is correct, the quantity of osmoregulant C known to accumulate within cells that are exposed to low water potentials should be sufficiently large to account for the amount of C released in the rewetting CO_2 pulse. If we assume the size of the rewetting CO_2 pulse (25 and 7 μg C-CO_2 g soil^{-1}, oak and grass soils respectively, see above) is roughly equivalent to the amount of cytoplasmic C mineralized and if we assume a biomass extraction efficiency (Kec) of 0.4 (Dictor et al., 1998), we can estimate that the quantity of cytoplasmic C mineralized in response to soil rewetting represents approximately 4 and 2% of the total microbial biomass C pools from oak and grass soils, respectively. Killham and Firestone (1984), studying the physiological response of two soil *Streptomyces* strains to salt stress, estimated that the total intracellular free amino acids (the primary osmoregulatory solutes in bacteria, Harris, 1981; Wood et al., 2001) increased by 1 mmol g cell (dry wt.)$^{-1}$ in response to a -5 MPa decrease in osmotic water potential. This allocation of C to osmoregulatory solutes represents approximately 10% of total cellular C. In a similar study with *Staphylococcus aureus*, a -8 MPa decrease in osmotic water potentials increased intracellular free amino acid concentrations by 1.75 mmol g cell (dry wt.)$^{-1}$, or approximately 20% of total cellular C (Koujima et al., 1978). Based on these general estimates, we can infer that only a fraction of the C accumulated as osmoregulatory solutes by cells under low water potential conditions would have to be mineralized to account for the CO_2 pulses generated by the rewetting of the dried oak and grass soils.

Since C is often the primary nutrient limiting microbial biomass growth in soil (Wardle, 1992), one might expect frequent drying and rewetting events to select for soil microbial communities that can adjust to sudden increases in water potential without rapidly mineralizing a large portion of their accumulated cytoplasmic solutes. Work by Halverson et al. (2000) has shown considerable variability in the physiological responses of different soil isolates to a rapid increase in water potentials. The additional mechanisms, besides mineralization, used by bacteria and fungi to remove accumulated osmoregula-

tory solutes if water potentials increase rapidly can include: the rapid polymerization or re-assimilation of the solutes into osmotically less active forms inside the cell (Halverson et al., 2000; Munro et al., 1972; Reed and Stewart, 1983), storage of the solutes in periplasmic space (Dylan et al., 1990), or leaving the solutes largely intact and having strong enough cell walls to sustain the large turgor pressures generated by the rapid increase in water potentials (Brown, 1990; Harris, 1981). Under natural conditions, grass soils are exposed to more frequent drying-rewetting events than oak soils (Parker and Muller, 1982). In this experiment, the rewetting CO_2 pulse from oak soils was three times larger than the pulse from grass soils (Fig. 2), even though the water potential increases were similar in magnitude. Some of this difference in the size of the rewetting CO_2 pulse may be a result of the microbial communities of the two soil types using different mechanisms to cope with the rapid increase in soil water potentials. We hypothesize that the microbes inhabiting oak soils largely reduce the activity of accumulated osmoregulatory solutes by mineralization, while the grass soil microbes utilize more efficient mechanisms to adjust intracellular water potentials, mineralizing a smaller proportion of their cytoplasmic C on rewetting.

Influence of Soil Drying and Rewetting on Soil Organic Matter-Carbon Pools

A drying-rewetting cycle will not only affect microbial biomass C pools, but can also alter the availability of SOM to soil microbes. The amount of extractable C increased following rewetting (Fig. 5a) and the low levels of enrichment (Fig. 5b) indicate that this C must be derived from SOM, not microbial biomass. Other studies have shown that a drying and rewetting process, by altering the structure of macro- and microaggregates, can render physically protected SOM extractable (Adu and Oades, 1978; Denef et al., 2001; Utomo and Dexter, 1982). In this experiment, the vigorous shaking associated with the extraction process disrupted the macroaggregate structures in all samples; therefore, the observed increase in extractable SOM can only be explained as a rewetting-induced disruption of microaggregate structures (<250 μm, Tisdall and Oades, 1982). A drying-rewetting cycle can break the bonds between soil particles or between clay leaves, releasing the SOM protected within microaggregates (Degens and Sparling, 1995; Denef et al., 2001; Pannabokke and Quirk, 1957). Over time, successive drying and rewetting events may therefore reduce the quantity of physically protected SOM in the soil.

With these soils, the additional SOM rendered extractable by the drying and rewetting process was not readily mineralized, as evidenced by the high ^{14}C enrichment of the CO_2 released after rewetting and the low enrichment of the extractable SOM-C. Related studies have also shown that the SOM-C released by drying and rewetting is likely to be stable and highly resistant to decomposition (Degens and Sparling, 1995; Lundquist et al., 1999a; Magid et al., 1999). Since recurrent drying and rewetting events should successively deplete the quantity of labile SOM held within aggregates (Degens and Sparling, 1995), soils that are rarely exposed to intense drying and rewetting events may release more labile SOM on experimental rewetting than the soils used in this study. Studies that have partially or wholly attributed a large CO_2 pulse after rewetting to an increase in SOM availability (Birch, 1961; Jager and Bruins, 1975; Pulleman and Tietema, 1999; Sorensen, 1974; Van Gestel et al., 1991), may have imposed a drying and rewetting treatment of greater intensity then that generally experienced by the soils under natural conditions, releasing physically protected SOM with a high degree of lability.

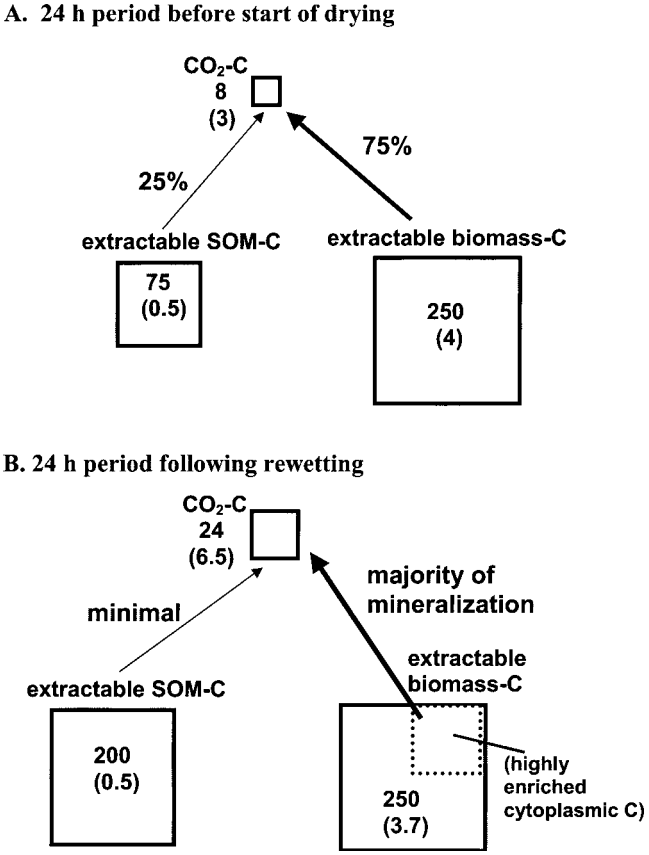

Fig. 6. A schematic diagram illustrating the changes in C pools before drying and after the rewetting of the dried soil. The carbon pools and the arrows showing the contribution of each of these C pools to total CO_2 production are conceptual representations of the hypotheses presented in this paper. Carbon pool size, in micrograms of C per gram of soil (μg C g soil^{-1})(values not in parentheses), and ^{14}C enrichment, in becquerels of 14C per gram of C (Bq ^{14}C g C^{-1})(values in parentheses), are indicated for each box. The size and enrichment of each C pool are estimated based on our measurements for the oak soil. The size of each box is equivalent to the estimated size of the carbon pool. We hypothesize that cytoplasmic solutes (indicated by the dashed box) are the major source of C mineralized after rewetting, however, the size and enrichment of this pool could not be directly measured.

SUMMARY

In Fig. 6, we present a schematic diagram summarizing the short-term influence of drying and rewetting on C pools in the oak soil. We propose that the rewetting

CO_2 pulse is generated from microbial biomass C, but without significant microbial cell lysis. Instead, the rewetting of a dry soil results in the rapid mineralization of cytoplasmic solutes for reasons, which, at this point, are still unclear. We can only hypothesize that the mineralized cytoplasmic solutes serve an osmoregulatory role inside cells, lowering intracellular water potentials during soil drying. At the onset of rewetting, these solutes are rapidly removed from cells so an equilibrium between intracellular and soil water potentials can be maintained. The rewetting of dried soil also increases the amount of SOM extractable from soil, but this additional SOM is not highly labile and does not contribute significantly to the rewetting CO_2 pulse. The conceptual model we present is based on experiments with two soils from a semi-arid, Mediterranean climate; qualitatively, the same mechanisms should apply to soils with different compositions and moisture stress histories, however, the magnitude of the rewetting CO_2 pulse may be highly variable.

In general, we would predict that soils which are rarely exposed to natural drying and rewetting cycles will exhibit a larger CO_2 pulse on rewetting than soils which are frequently dried and rewet: a greater proportion of the C in osmoregulatory solutes will be mineralized by cells on rewetting and any additional SOM made accessible to microbes by the drying and rewetting process will be more labile. If proven correct, this proposed scenario may explain why the rewetting CO_2 pulse is often reduced in size after a soil has been exposed to numerous drying and rewetting cycles (Birch, 1958; Bottner, 1985; Denef et al., 2001; Fierer and Schimel, 2002; Sorensen, 1974) and why soils collected from areas with a high degree of moisture variability tend to have smaller CO_2 pulses on rewetting compared with soils which rarely experience large fluctuations in moisture (Franzluebbers et al., 2000; Kieft et al., 1987; Lundquist et al., 1999b; Van Gestel et al., 1993a).

ACKNOWLEDGMENTS

We thank Andrew Allen for his considerable help in the planning and implementation of this experiment. We also thank Carl Mikan and Allen Doyle for assistance with laboratory methods. Financial support for this work was provided the NSF Microbial Observatories Program (MCB-9977874) and the UC Campus-Laboratory Collaboration Program.

REFERENCES

Adu, J., and J. Oades. 1978. Physical factors influencing decomposition of organic materials in soil aggregates. Soil Biol. Biochem. 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 Biol. Biochem. 30:1445–1456.

Barrow, E., and M. Hulme. 1996. Changing probabilities of daily temperature extremes in the UK related to future global warming and changes in climate variability. Climate Res. 6:21–31.

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

Birch, H.F. 1961. Resistance of humus to decomposition. Nature (London) 4789:731–732.

Bloem, J., P.D. Ruiter, G. Koopman, G. Lebbink, and L. Brussaard. 1992. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. Soil Biol. Biochem. 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 Biol. Biochem. 17:329–337.

Bremer, E., and C. Van Kessel. 1990. Extractability of microbial ^{14}C and ^{15}N following addition of variable rates of labelled glucose and $(NH_4)_2SO_4$ to soil. Soil Biol. Biochem. 22:707–713.

Bremer, E., and P. Kuikman. 1994. Microbial utilization of ^{14}C glucose in soil is affected by the amount and timing of glucose additions. Soil Biol. Biochem. 26:511–517.

Brown, A. 1990. Microbial water stress physiology: Principles and perspectives. John Wiley and Sons, Ltd., Chichester, UK.

Cabrera, M.L., and M.H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57:1007–1012.

Clein, J., and J. Schimel. 1994. Reduction in microbial activity in birch litter due to drying and rewetting events. Soil Biol. Biochem. 26: 403–406.

Csonka, L., and A. Hanson. 1991. Prokaryotic osmoregulation: Genetics and physiology. Annu. Rev. Microbiol. 45:569–606.

Degens, B.P., and G.P. Sparling. 1995. Repeated wet-dry cycles do not accelerate the mineralization of organic C involved in the macro-aggregation of a sandy loam soil. Plant Soil 175:197–203.

Dictor, M., L. Tessier, and G. Soulas. 1998. Reassessement of the Kec coefficient of the fumigation-extraction method in a soil profile. Soil Biol. Biochem. 30:119–127.

Denef, K., J. Six, H. Bossuyt, S.D. Frey, E.T. Elliott, R. Merckx, and K. Paustian. 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol. Biochem. 33:1599–1611.

Dylan, T., D.R. Helinski, and G.S. Ditta. 1990. Hypoosmotic adaptation in *Rhizobium meliloti* requires B-(1–2)-glucan. J. Bacteriol. 172:140–1408.

Fierer, N., and J. Schimel. 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biol. Biochem. 34:777–787.

Franzluebbers, A., R. Haney, C. Honeycutt, H. Schomberg, and F. Hons. 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J. 64:613–623.

Gessler, P.E., O.A. Chadwick, F. Chamran, L. Althouse, and K. Holmes. 2000. Modeling soil-landscape and ecosystem properties using terrain attributes. Soil Sci. Soc. Am. J. 64:2046–2056.

Gregorich, E.G., G. Wen, R.P. Voroney, and R.G. Kachanoski. 1990. Calibration of a rapid direct chloroform extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 22: 1009–1011.

Halverson, L.J., T.M. Jones, and M.K. Firestone. 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci. Soc. Am. J. 64:1630–1637.

Harris, R. 1981. Effect of water potential on microbial growth and activity, p. 23–95. *In* J. Parr et al. (ed.) Water potential relations in soil microbiology. SSSA Spec. Pub. 9, SSSA, Madison, WI.

Hohmann, S. 2002. Osmotic stress signaling and osmoadaptation in yeasts. Microbiol. Mol. Biol. Rev. 66:300–372.

Houghton, J., L.M. Filho, B. Callander, N. Harris, A. Lattenberg, and K. Maskell. (ed.) 1996. Climate change 1996: The science of climate change. Contribution of Working Group I to the Second Assessment Report of the Intergovernmental Panel on Climate Change, p. 1–364. Cambridge University Press, Cambridge.

Jager, G., and E. Bruins. 1975. Effect of repeated drying at different temperatures on soil organic matter decomposition and characteristics, and on the soil microflora. Soil Biol. Biochem. 7:153–159.

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

Killham, K., and M. Firestone. 1984. Salt stress control of intracellular solutes in *Streptomyces* indigenous to saline soils. Appl. Environ. Microbiol. 47:301–306.

Koujima, I., H. Hayashi, K. Tomochika, A. Okabe, and Y. Kanemasa. 1978. Adaptational change in proline and water content of *Staphylococcus aureus* after alteration of environmental salt concentration. Appl. Environ. Microbiol. 35:467–470.

Lundquist, E., L. Jackson, and K. Scow. 1999a. Wet dry cycles affect DOC in 2 California agricultural soils. Soil Biol. Biochem. 31: 1031–1038.

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

Magid, J., C. Kjaergaard, A. Gorissen, and P. Kuikman. 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 Biol. Biochem. 31:595–602.

Munro, G., K. Hercules, J. Morgan, and W. Sauerbier. 1972. Dependence of the putrescine content of *Escherichia coli* on the osmotic strength of the medium. J. Biol. Chem. 247:1272–1280.

Pannabokke, C., and J. Quirk. 1957. Effect of initial water content on stability of soil aggregates in water. Soil Sci. 83:185–195.

Parker, V.T., and C.H. Muller. 1982. Vegetational and environmental changes beneath isolated live oak trees (*Quercus agrifolia*) in a California annual grassland. Am. Midland Nat. 107:69–81.

Potts, M. 1994. Desiccation tolerance of prokaryotes. Microbiol. Rev. 58:755–805.

Pulleman, M., and A. Tietema. 1999. Microbial C and N transformations during drying and rewetting of coniferous forest floor material. Soil Biol. Biochem. 31:275–285.

Reed, R., and W. Stewart. 1983. Physiological responses of *Rivularia atra* to salinity: Osmotic adjustment in hyposaline media. New Phytol. 95:595–603.

Ruffert, S., C. Lambert, H. Peter, V.F. Wendisch, and R. Kraemer. 1997. Efflux of compatible solutes in *Corynebacterium glutamicum* mediated by osmoregulated channel activity. Eur. J. Biochem. 247:572–580.

Scheu, S., and D. Parkinson. 1994. Changes in bacterial and fungal biomass C, bacterial and fungal biovolume and ergosterol content after drying, remoistening and incubation of different layers of cool temperate forest soils. Soil Biol. Biochem. 26:1515–1525.

Schleyer, M., R. Schmid, and E.P. Bakker. 1993. Transient, specific and extremely rapid release of osmolytes from growing cells of *Escherichia coli* K-12 exposed to hypoosmotic shock. Arch. Microbiol. 160:424–431.

Sleator, R., and C. Hill. 2001. Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. FEMS Microbiol. Rev. 25:49–71.

Sorensen, L. 1974. Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated additions of organic material. Soil Biol. Biochem. 6:287–292.

Soulides, D., and F. Allison. 1961. Effect of drying and freezing soils on carbon dioxide production, available mineral nutrients, aggregation, and bacterial population. Soil Sci. 91:291–298.

SPSS, Inc. 2000. Systat for windows. Release 10. SPSS Inc., Evanston, IL.

Tisdall, J., and J. Oades. 1982. Organic matter and water stable aggregates in soils. J. Soil Sci. 33:141–163.

Tschicholz, I., and H. Truper. 1990. Fate of compatible solutes during dilution stress in *Ectothiorhodospira halochloris*. FEMS Microbiol. Ecol. 73:181–186.

Utomo, W., and A. Dexter. 1982. Changes in soil aggregate water stability induced by wetting and drying cycles in non-saturated soil. J. Soil Sci. 33:623–637.

Van Gestel, M., J. Ladd, and M. Amato. 1991. Carbon and nitrogen mineralization from two soils of contrasting texture and microaggregate stability: Influence of sequential fumigation, drying, and storage. Soil Biol. Biochem. 23:313–322.

Van Gestel, M., R. Merckx, and K. Vlassak. 1993a. Microbial biomass and activity in soils with fluctuating water contents. Geoderma 56:617–626.

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

Van Veen, J., J. Ladd, and M. Amato. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with 14-C glucose and 15-N ammonium sulfate under different moisture regimes. Soil Biol. Biochem. 17:747–756.

Waggoner, P. 1989. Anticipating the frequency distribution of precipitation if climate change alters its mean. Agric. For. Meteorol. 47: 321–337.

Wardle, D.A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. Cambridge Phil. Soc. 67:321–358.

Wood, J.M., E. Bremer, L.N. Csonka, R. Kraemer, B. Poolman, T. van der Heide, and L.T. Smith. 2001. Osmosensing and osmoregulatory compatible solute accumulation by bacteria. Comp. Biochem. Physiol. Part A. 130A:437–460.