The ISME Journal (2009) 3, 1325–1334

www.nature.com/ismej

ORIGINAL ARTICLE

Water stress impacts on bacterial carbon monoxide oxidation on recent volcanic deposits

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Water availability oscillates dramatically on young volcanic deposits, and may control the distribution and activity of microbes during early stages of biological succession. Carbon monoxide (CO)-oxidizing bacteria are among the pioneering colonists on volcanic deposits and are subjected to these water stresses. We report here the effects of water potential on CO-oxidizing bacteria in unvegetated (bare) and vegetated (canopy) sites on a 1959 volcanic deposit on Kilauea Volcano (Hawai'i). Time course measurements of water potential showed that average water potentials in the surface layer (0–1 cm) of canopy soil remained between −0.1 and 0 MPa, whereas dramatic diurnal oscillations (for example, between −60 and 0 MPa) occur in bare site surface cinders. During a moderate drying event *in situ* (−1.7 to 0 MPa), atmospheric CO consumption by intact bare site cores decreased 2.7-fold. For bare and canopy surface samples, maximum potential CO oxidation rates decreased 40 and 60%, respectively, when water potentials were lowered from 0 to −1.5 MPa in the laboratory. These observations indicated that CO oxidation is moderately sensitive to changes in water potential. Additional analyses showed that CO oxidation resumes within a few hours of rehydration, even after desiccation at −150 MPa for 63 days. Samples from both sites exposed to multiple cycles of drying and rewetting (−80 to 0 MPa), lost significant activity after the first cycle, but not after subsequent cycles. Similar responses of CO oxidation in both sites suggested that active CO-oxidizing communities in bare and canopy sites do not express differential adaptations to water stress.
The ISME Journal (2009) **3,** 1325–1334; doi:10.1038/ismej.2009.70; published online 30 July 2009
Subject Category: microbial ecology and functional diversity of natural habitats
Keywords: biogeochemistry; water potential; desiccation tolerance; carbon monoxide; volcanic; physiology

Introduction

Changes in water availability, measured as water potential, can substantially alter microbial communities and their activity. For example, T-RFLP patterns for 16S rRNA genes changed after drying oak soils, but not in grassland soils (Fierer *et al.*, 2003). As the latter experienced more frequent oscillations in water status than the former, the results indicated that water stress may have a function in the structure of the inherently different microbial communities in these two soils (Fierer *et al.*, 2003). Other studies have also shown that microbial communities in grassland soils are resistant to water stress (Griffiths *et al.*, 2003).

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Received 9 April 2009; revised 18 May 2009; accepted 21 May 2009; published online 30 July 2009

Rapid drying or decreases in water potential allow little time for acclimation, and adversely impact cell viability (Potts, 1994). The effects of changing water potential can be exacerbated by extreme temperatures, low substrate availability and other physiological stresses (Mary *et al.*, 1985; Kieft *et al.*, 1987; Potts, 1994). Rapid water loss causes macromolecular and cellular destabilization, which inhibit enzyme activity and induce production of reactive oxygen species and DNA damage (Potts, 1994). Cellular defenses against desiccation-induced damage include accumulation of compatible solutes, exopolysaccharide production and enzyme synthesis to combat oxidative stress (Singh *et al.*, 2005; Leblanc *et al.*, 2008).

Likewise, rapid rehydration can adversely impact cell viability. In this case, cells must reduce compatible concentrations to avoid lysis because of elevated turgor pressure (Potts, 1994). Several studies have documented rehydration as a significant source of cell lysis and carbon turnover in soils (Bottner, 1985; Van Gestel *et al.*, 1993; Grierson *et al.*, 1998; Magid *et al.*, 1999; Turner and Haygarth, 2001;

Turner *et al.*, 2003; Wu and Brookes, 2005; Schimel *et al.*, 2007).

Water availability can oscillate dramatically on unvegetated volcanic deposits, and likely has an important function in regulating the activity and distribution of pioneering microbial colonists. Regardless of age, unvegetated deposits are often coarse and porous with little buffering capacity against changes in water status. The lack of buffering capacity likely results from a combination of the texture and lack of organic matter (Rawls *et al.*, 2003).

Despite limitations imposed by water stress as well as limited substrate availability, a variety of bacteria colonize and are active on young unvegetated volcanic deposits. Carbon monoxide (CO)-oxidizing bacteria are among the earliest successful colonists. A field study by King and Weber (King, 2007) revealed that unvegetated, fresh lava chips supported measurable CO oxidation within 6 months. Additional studies have shown that atmospheric CO consumption accounts for 2–10% of reducing equivalent flow for Kilauea volcanic deposits (King, 2003; King and Weber, 2008). Comparable rates of CO uptake have been observed on 23-year-old volcanic deposits in Miyake-jima, Japan (King *et al.*, 2008). Nonetheless, CO oxidizers, like other functional groups, must respond to local water regimes, but there are no published studies that document the response of CO oxidizers to *in situ* water regimes.

Plant colonization seems to have a major function in water regimes for Kilauea deposits. Plant colonization promotes weathering and organic matter accumulation, which increases water retention and results in more stable and less stressful water potentials. This may lead to a shift over time from water stress-tolerant to water stress-sensitive phenotypes. Little is known, however, about the impacts of water stress on microbial succession, and in particular how water stress impacts CO-oxidizer community development and adaptation.

Very few studies have dealt with the impacts of soil water status on CO-oxidizer activity (Spratt and Hubbard, 1981; Moxley and Smith, 1998; King, 1999a). Results from these studies suggest that CO oxidation is water sensitive, but that CO oxidizers may tolerate and even adapt to dry conditions. Spratt and Hubbard (1981) measured optimal CO consumption when soils were incubated under relative humidities approaching 100%. Significant increases in CO consumption were observed, however, in soils that had been air-dried and subsequently rewetted or equilibrated at relative humidities >93%. Moxley and Smith (1998) found that the optimal CO-oxidation rates of three Scottish soils occurred at water contents that were about field capacity. In the same study, two arable soils had optimal CO-consumption rates at water contents that were lower than a woodland soil (10–15 vs 25–30%). The authors suggested that the CO-oxidizing communities in each soil type may be adapted to these different water contents.

King (1999a) examined CO consumption by Maine forest 'O'-horizon soils as they were dried under laboratory conditions and then gradually rewetted by step-wise additions of deionized water. Soils produced CO when dried to water contents below 20%. On rewetting, a hysteresis was observed for water contents of 20–80%. King (1999a), however, noted that limited drying and wetting cycles, which might better represent what occurs *in situ*, seemed to have no inhibitory effect on CO oxidation. This observation provides the first insights that CO oxidation may be somewhat resilient to *in situ* water dynamics in forest soils (King, 1999a).

In this study, we examined the impacts of water stress on CO-oxidizer activity at two sites representing early and late successional stages on a 1959 deposit on Kilauea Volcano (Hawai'i). The two sites differ dramatically in water regimes with one site exhibiting diurnal oscillations in water potential (bare site) and the other site remaining relatively moist (canopy site). Earlier molecular ecological surveys at these two sites have revealed distinct CO-oxidizer communities (Weber and King, submitted). The objectives of this study were to: (1) determine the impact of decreased water potential on CO-oxidation rates, (2) assess the ability for CO oxidation to recover after drying and rewetting events, and (3) determine whether the activity of distinct communities at the two sites show differential adaptations to local water regimes.

Materials and methods

Site descriptions

Volcanic material used in this study was collected from the Pu'u Puai deposit, which resulted from a 1959 eruption of Kilauea Iki (Kilauea Volcano, Hawai'i; GPS coordinates: 19° 24′ 22.5″ N X 155° 15′ 18.2″ W). A deposit several meters thick and comprised of cinders averaging about 1 cm in diameter, supported 'tree islands', which are irregularly shaped vegetated patches typically $>100\,m^2$ consisting largely of *Meterosideros polymorpha* (the Ohia lehua tree) and *Morella faya* (also known as *Myrica faya*; fire tree). Sites designated 'bare' were comprised of unvegetated cinders and those designated 'canopy' were comprised of an organic-rich soil, which has accumulated on top of and within the original cinder deposit within tree islands (see Supplementary Information). A 5–10 cm thick litter layer overlies the soil in canopy sites and was removed before all sampling. Canopy and bare sites were located within 10 m of each other. Physical and chemical characteristics of the materials at these sites as well as the composition of CO-oxidizing and bacterial communities have been described earlier (King and Weber, 2008; Weber and King, submitted).

Spatial and temporal variability: relative humidity, water potential and CO uptake

Relative humidity (RH) and temperature profiles were obtained using U-series external channel HOBO Data Loggers (Onset Computer Corp.; Pocasset, MA, USA) equipped with TMC50-HD soil temperature sensors. RH was measured at a height of about 1.5 m at each site. RH profiles were obtained during 6–7 October 2007, 28–30 January 2008 and 3–7 May 2008. During these periods, triplicate surface samples (upper 1 cm) were collected from the bare and canopy sites at regular intervals for water potential measurements. Samples were returned to the field laboratory at ambient temperature and RH in open containers to prevent alterations in water potential during transport. Samples were visually inspected to make sure that no condensation was formed on the sides of the collection containers. On arriving at the laboratory (no more than 20 min after field-collection), ~0.5–1 g fresh weight (gfw) samples were placed into 14 ml sample cups (Decagon Devices, Pullman, WA, USA) in which water potentials were measured using a WP4-T dewpoint potentiometer (Decagon Devices, Pullman, WA, USA) according to the manufacturer's instructions. This instrument measures water potentials ranging from 0 to −300 MPa. At each sampling, ambient RH was measured using a Kestrel 4000 (Forestry Suppliers; Jackson, MS, USA), a handheld instrument equipped with RH and temperature sensors. Surface temperatures (top 1 cm) at both sites were recorded at 10-min intervals using a HOBO data logger.

To determine the distribution of water and maximum potential CO uptake activity in the upper 15 cm of the bare sites, two sets of triplicate bare site cores were collected in the morning (about 0800) and afternoon (about 1600) on 5 May 2008. Cores were collected using aluminum core tubes (7.2 cm dia) that had been ethanol-cleaned and baked dry in an oven. Cores were sectioned into the following depth intervals: 0–1, 1–3, 3–5, 5–8, 8–11 and 11–15 cm. Immediately after sectioning, ~5-gfw samples of each fraction were transferred to 110 cm^3 jars. Jar headspaces were spiked with CO to a final concentration of about 50 p.p.m. In all, 1 cm^3 volumes were removed at regular intervals and CO concentrations were determined using a Trace Analytical RGD Gas Chromatograph as described earlier (King, 1999b). CO uptake rates were determined using curve fitting procedures as described earlier (King, 1999b). Rates were normalized per gram dry weight (gdw).

To determine the impacts of *in situ* drying on CO uptake at ambient concentrations by intact bare cores and the water distributions in the upper 15 cm, triplicate intact cores were collected as above in the morning and the afternoon of 7 May 2008. CO uptake assays were initiated no more than 30 min after collection. Core tubes were sealed with gas-tight plastic caps with rubber septa sampling ports. After sealing the core tubes, 18 cm^3 of ambient air were added to the headspaces to provide an overpressure that would accommodate multiple headspace samplings. At regular intervals, 3 cm^3 samples were removed and CO concentrations were determined as described earlier (King, 1999b). Immediately following CO uptake rate analyses, cores were sectioned into the depth intervals described above for water potential and water content measurements. The water contents were determined after drying samples in the oven overnight at 176 °C.

Response of CO oxidation to polyethylene glycol 200 amendment

Maximum potential CO uptake rates were determined for triplicate 10-gfw samples of canopy surface soil (0–1 cm) that were amended with 2 ml of sterile water or solutions of 50, 75 or 100% polyethylene glycol 200 (PEG 200), which resulted in water potentials of 0, −0.85, −1.50 and −2.37 MPa, respectively. Triplicate 20–21-gfw samples of bare surface cinders (0–1 cm depth interval) were amended with 3 ml of water or solutions of 16, 32 or 50 % PEG 200, which resulted in water potentials of 0, −0.49, −1.25 and −2.77 MPa, respectively. After amending soil and cinders with water or PEG 200, samples were transferred into 500 cm^3 gas tight jars and allowed to equilibrate for 1 h. Jar headspaces were amended with CO to a final concentration of ~80 p.p.m. with enough overpressure to accommodate multiple headspace samplings. Headspace concentrations were assayed immediately and then measured at appropriate intervals thereafter as described above. After completing the CO uptake assay, samples were removed from the jars to determine the water content and dry weight of the samples.

Response of CO oxidation to long-term desiccation and rehydration

CO uptake by freshly collected bare cinders (water content: 29%; 0 MPa) and canopy soil (water content: 76%; 0 MPa) was measured as described earlier (King, 1999b). Briefly, triplicate 6–6.5-gfw samples of canopy soil and triplicate 7–8-gfw samples of bare cinders were placed into 110 cm^3 gas-tight jars and amended with CO to a final concentration of 70–80 p.p.m. with suitable overpressure to accommodate multiple headspace samplings. Headspace concentrations were determined immediately and at suitable intervals thereafter. From the same soil and cinder collections, 12 additional samples of cinders and canopy soil were placed into WP4-T sample cups, and then placed into a desiccator for drying. Samples were desiccated to water potentials of −150 MPa, which were maintained for the duration of the experiment. At intervals of 14, 27, 38 and 63 days, triplicate samples were rehydrated by applying 2 ml of sterile deionized water and gently mixing with a sterile

spatula. Immediately after rehydration, samples were placed into 110 cm^3 gas-tight jars. Jar headspaces were amended with CO to concentrations of 70–80 p.p.m. Headspace concentrations were determined immediately and at suitable intervals thereafter. Seventy-five days after initiating the experiment, CO uptake assays were carried out as described above for triplicate bare and canopy samples that had been stored at field water content and potential in ziptop bags.

Response of CO oxidation to oscillating water regimes
Six samples of bare cinders (13–15.5 gfw each) and canopy soil (6–8.5 gfw each) were transferred into 110 cm^3 jars and amended with CO to a final concentration of 60 p.p.m. with suitable overpressure to accommodate multiple headspace samplings. After the CO uptake assay, samples were weighed and then dried at ambient room temperature and RH (same as above) to a water potential of ~ -80 MPa. The dry masses of the samples were measured to determine water contents. After 2–8 days, samples were rehydrated with 2 ml of sterile deionized water to a water potential near 0 MPa (water contents: 27% [bare]; 66% [canopy]) and placed into 110 cm^3 jars to determine the CO uptake rates as before. After the CO uptake assay was completed, samples were re-weighed and dried again. Four additional cycles of drying and rewetting with CO uptake rate determinations were completed as above.

Statistical analyses
For the experiments examining the response of PEG amendment or long-term desiccation and rehydration on CO-oxidation rates in bare and canopy samples, average rates of CO uptake for each treatment and control group were statistically analysed by an analysis of variance. Differences among treatments were assessed using a Tukey's Honest Significant Difference test using KaleidaGraph Software (Synergy Software, Reading PA, USA). Water potentials and water contents of depth intervals in bare cores and whole core CO-oxidation rates were statistically examined in a similar manner. The responses of CO oxidation in bare and canopy samples to multiple cycles of desiccation and rehydration were examined statistically using a repeated-measures analysis of variance.

Results

Spatial and temporal variability: RH, water potential and CO uptake
Ambient temperature, cinder and soil surface temperatures and relative humidities varied diurnally at bare and canopy sites. Diurnal variations in ambient temperatures were always larger in the bare site than in the canopy site. The average low and high ambient temperatures observed during October 2007, January 2008 and May 2008 at the bare site were 13.2 ± 1.6 and 25.2 ± 0.8 °C, respectively, and those observed at the canopy site were 12.7 ± 1.5 and 19.4 ± 1.6 °C, respectively. Surface temperatures at the two sites also followed this trend, but the difference in the average maximum surface temperatures for the two sites was much more dramatic. The average low and high surface temperatures observed at the bare site were 13.8 ± 2.1 and 40.6 ± 2.6 °C, respectively, and those observed for the canopy site were 13.1 ± 1.6 and 18.4 ± 1.8 °C, respectively. RH oscillated across a similar range for the two sites (bare site: 50.8 ± 7.7–88.8 ± 0.4%; canopy site: 55.1 ± 8.2–89.2 ± 2.9%) with lower values typically during mid-day (Figure 1).

During a relatively dry period (October 2007), water potentials at the bare site decreased dramatically, dropping from near 0 MPa in the early morning to -60 ± 16 MPa by mid-day and increasing to -6.3 ± 1.1 MPa a few hours later (Figure 1). In contrast, during this interval, average water potentials in the canopy surface never dropped below 0 MPa. During wetter periods (January and May 2008), diurnal fluctuations in RH and water potential were smaller than in October 2007, but bare site water potentials still decreased during the day to values as low as -3.7 ± 1.4 MPa (January 2008) to -7.4 ± 2.4 MPa (May 2008).

Water potentials and contents as well as maximum CO uptake rates did not differ significantly between the two sets of cores collected in the morning and afternoon on 5 May 2008. Water potentials in the upper 3 cm ranged from near 0 to -0.33 MPa, whereas all water potentials at depths below 3 cm were near 0 MPa (Table 1). Water contents increased slightly with depth (Table 1). Maximum CO uptake rates were most variable, but highest for the 0–1 cm depth interval (morning: 62 ± 25 nmol gdw^{-1} $days^{-1}$; afternoon: 190 ± 98 nmol gdw^{-1} $days^{-1}$) and decreased at lower depths (Table 1).

Two additional sets of triplicate cores were collected at 1030 and 1400 on 7 May 2008. The first set of cores was moist from recent rainfall. Water content was lowest in the surface (12.5 ± 0.6%) and increased with depth to 36.9 ± 1.9% in the 8–11 cm depth interval (Table 2). Average water potentials were near 0 MPa for all depth intervals assayed in the first set of cores (Table 2). The second set of cores was drier than the first. Average water contents for the 0–1 and 1–3 cm layers of the afternoon cores were significantly lower than those of morning cores ($P = 0.0006$, $P = 0.027$, respectively), with the water contents of the 0–1 and 1–3 cm depth intervals dropping to 2.0 ± 0.8 and 11.0 ± 0.8%, respectively (Table 2). Likewise, water potentials for the 0–1 cm depth interval of the afternoon cores (-1.73 ± 0.6 MPa) were significantly lower ($P = 0.0002$) than water potentials for the morning cores (-0.04 ± 0.04 MPa). Average ambient atmospheric CO uptake rates for the afternoon cores

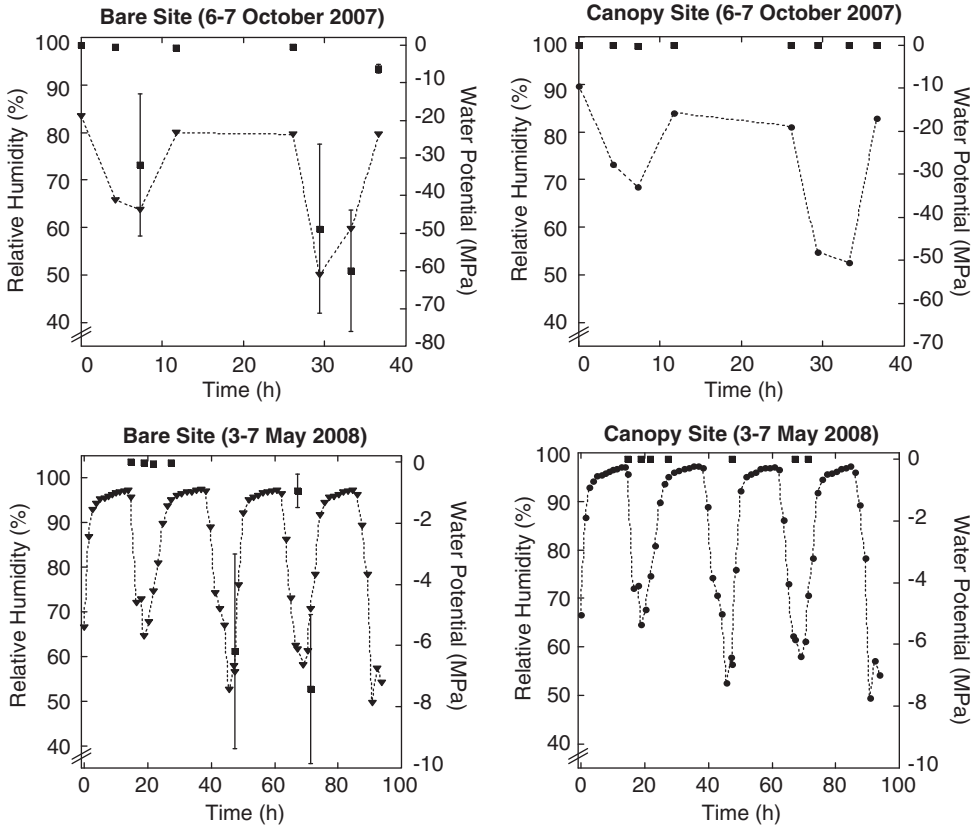

Figure 1 Relative humidity (canopy = ●, bare = ▼) and water potentials (■) bare and canopy sites for 6–7 October 2007 (beginning at 0700) and 3–7 May 2008 (beginning at 1600). Water potentials are averages of triplicate samples ± 1 s.e. Note the difference in the *Y* axis (water potential) for the October 2007 and May 2008 plots.

Table 1 Water content (%), water potential (MPa) and maximum potential CO uptake rates (nmol gdw^{-1} day^{-1}) for two sets of triplicate bare site cores collected at 0800 (A) and 1600 (B) on 5 May 2008

Core set (bare)	*A*	*B*	*A*	*B*	*A*	*B*
Depth	*Water content*		*Water potential*		*Maximum uptake*	
0–1 cm	13.2 (1.7)	10.5 (2.3)	−0.02 (0.02)	−0.20 (0.02)	62 (25)	190 (98)
1–3 cm	17.1 (5.8)	20.5 (0.8)	−0.16 (0.10)	−0.03 (0.03)	71 (12)	68 (16)
3–5 cm	32.5 (1.3)	31.8 (1.4)	0 (0)	0 (0)	43 (13)	46 (6)
5–8 cm	32.9 (1.7)	31.1 (0.5)	0 (0)	0 (0)	22 (5)	22 (2)
8–11 cm	36.0 (1.9)	35.3 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)
11–15 cm	30.0 (2.6)	27.2 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)

(1.8 ± 0.8 mg m^{-2} days^{-1}) were also lower than rates for the morning cores (5.0 ± 2.2 mg m^{-2} days^{-1}), but the rates for the two sets were not statistically different.

CO-oxidation response to in vitro *water potential manipulation*

Maximum potential CO-oxidation rates in bare and canopy samples were reduced substantially in response to lowered matric potentials adjusted by amendments with PEG 200. At water potentials ranging from −1.25 to −1.5 MPa, CO-oxidation rates in the bare and canopy surface materials were reduced to 40 and 60% of water-amended controls, respectively (Figure 2).

Rates of CO oxidation in bare samples differed significantly from the water-amended controls at water potentials ranging from −1.25 to −2.77 MPa ($P = 0.008$ and 0.0209; Figure 2), but rates of the three PEG 200 treatments did not differ significantly from one another ($P > 0.35$). Rates of CO oxidation in canopy samples at water potentials of −0.92 to −2.03 MPa were significantly lower than the water-

Table 2 Average water potential (MPa), water content (%) and CO uptake rates ($mg\,m^{-2}\,day^{-1}$) at ambient CO concentrations for triplicate cores collected from the bare site in the morning and afternoon on 7 May 2008

Core section	*Time*			
	1030		*1600*	
	Water potential	*Water content*	*Water potential*	*Water content*
0–1 cm	−0.04 (0.04)	12.5 (0.6)	−1.73 (0.6)	2.0 (0.8)
1–3 cm	0 (0)	18.2 (1.0)	−0.35 (0.08)	11.0 (0.8)
3–5 cm	0 (0)	29.4 (1.5)	−0.01 (0.01)	26.6 (0.6)
5–8 cm	0 (0)	36.1 (2.2)	0 (0)	34.7 (0.6)
8–11 cm	0 (0)	36.9 (1.9)	0 (0)	33.9 (1.9)
11–15 cm	0 (0)	32.0 (3.1)	0 (0)	33.4 (1.5)
CO uptake rate	5.0 (2.2)		1.8 (0.8)	

Numbers in parentheses are standard errors.

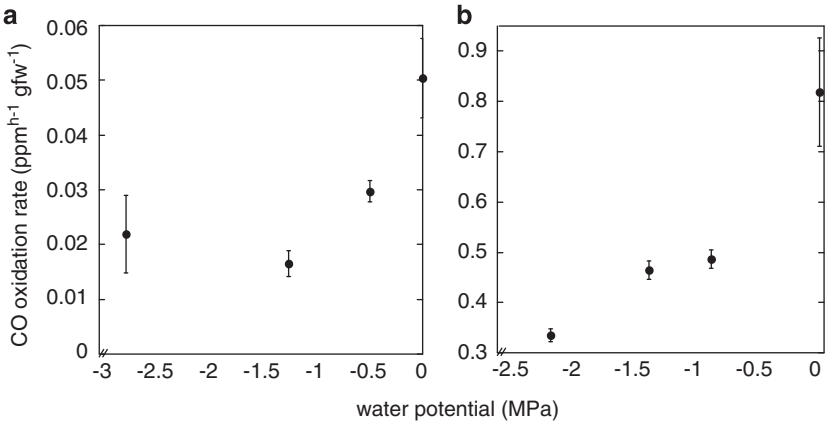

Figure 2 CO-oxidation rates of PEG 200 amended (**a**) bare and (**b**) canopy samples and water-amended controls. Data points are averages of triplicate samples ± 1 s.e.

amended control (all *P*-values $\leqslant 0.0267$), but rates of the PEG 200-amended treatments did not differ significantly from one another (all *P*-values $\geqslant 0.3391$).

To determine the resilience of CO-oxidizing communities in bare and canopy surface materials to extended desiccation, maximum potential CO uptake was measured in samples that had been rehydrated after desiccation for 14, 27, 39 or 63 days. Maximum CO uptake rates for material stored at field water contents and potentials did not vary significantly over the duration of the incubation (bare: $P = 0.624$; canopy: $P = 0.998$). Non-desiccated canopy sample rates were significantly greater than rates of all desiccated and rehydrated samples ($P < 0.0001$; Figure 3). Rates of desiccated and rehydrated samples did not differ statistically from one another ($P > 0.05$). Similar patterns were observed for bare site samples (Figure 3).

To determine the impact of multiple oscillations in water status on CO oxidation, maximum potential CO uptake rates were measured in surface samples subjected to five cycles of desiccation to −80 MPa and rehydration to 0 MPa. Before desiccation, bare and canopy water potentials were 0 MPa with water contents of 33 and 84%, respectively; these bare and canopy samples oxidized CO at rates of 58 ± 2.5 and $840 \pm 37.5\,nmol\,gdw^{-1}\,days^{-1}$, respectively (Figure 4). After one cycle of desiccation and rehydration, activity in bare and canopy samples was reduced to 55 and 50% of the initial rates, respectively; these decreases were statistically significant ($P < 0.001$; Figure 4). For bare samples, CO-oxidation rates increased through cycle 4 and decreased after cycle 5, but these changes were not statistically significant (Figure 4). Similar patterns were observed for canopy samples.

Discussion

In situ water dynamics in the canopy and bare sites differ markedly. Canopy surface water potentials remain at or very near 0 MPa; in contrast, bare site surface cinders experience diurnal shifts in water potential that would inhibit activities of even some of the most stress-tolerant bacteria (that is, −5 MPa;

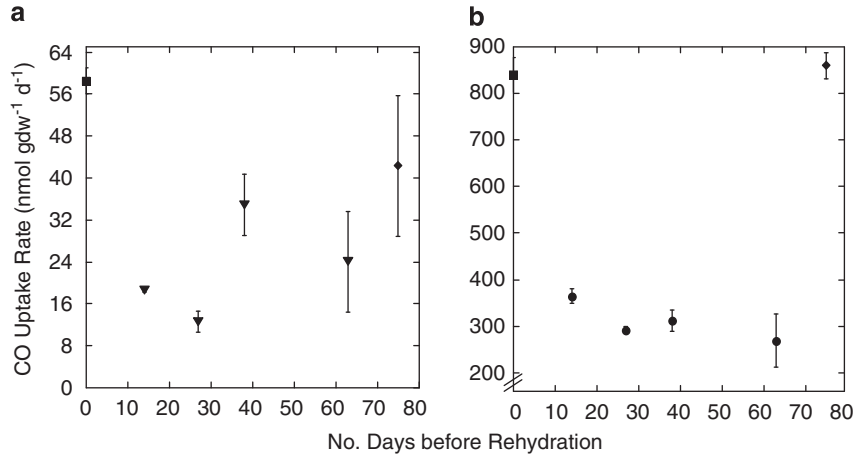

Figure 3 CO-oxidation response on rehydration of (**a**) bare and (**b**) canopy materials after 14, 27, 39 or 63 days storage at −150 MPa. Rates of non-desiccated controls before the experiment (■) and after the experiment (◆). Data points are averages of triplicate samples ± 1 s.e. Note the scale differences in the *Y* axis.

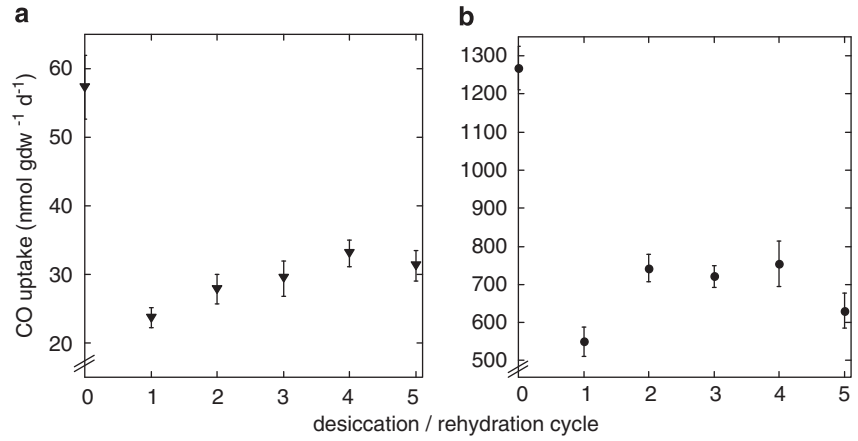

Figure 4 CO-oxidation response to multiple cycles of desiccation to −80 MPa and rehydration to 0 MPa in (**a**) bare and (**b**) canopy samples. Rates are averages of triplicate samples ± 1 s.e. plotted as a function of the number of desiccation/rehydration cycles. Note the scale differences in the *Y* axis on the two plots.

Griffin, 1981). Accordingly, CO uptake rates by intact bare cores were higher for a morning sampling relative to an afternoon sampling during a period of moderate drying *in situ* (Table 2). This trend is consistent with observations of past studies that suggest that CO-oxidizer activity is sensitive to water stress (King, 1999a; Moxley and Smith, 1998).

Maximum potential CO uptake by canopy and bare samples at various matric stresses adjusted using PEG 200 clearly showed sensitivity to changes in water potential and content. Although the efficacy of using PEG as a proxy for matric stress imposed by drying has been questioned, recent transcriptomic work on *Bradyrhizobium japonicum* shows that responses to matric stresses imposed by desiccation and PEG amendment are remarkably similar (Cytryn *et al.*, 2007). Therefore, significant decreases in CO uptake rates in response to PEG amendment are likely representative of responses of CO oxidizers to matric stresses.

Even though CO oxidation is sensitive to water stress, results from additional experiments demonstrated that it recovers rapidly from extended periods of desiccation. Bare and canopy surface samples that had been stored at a water potential of −150 MPa resumed rapid activity usually within 2 h after rehydration to 0 MPa, even after storage for 63 days. This indicates that despite the different water regimes at the two sites, both communities have core populations that withstand and recover from severe water stress. These findings are similar to those noted for Maine forest soils, in which activity resumed after soils were dried to water contents $<20\%$ (King, 1999a).

CO-oxidizer activity in bare and canopy sites also responded similarly when exposed to multiple cycles of desiccation and rehydration. After one cycle of desiccation and rehydration, activity for both sites was significantly reduced, but subsequent cycles of drying and rewetting had little effect, and

CO-oxidation rates even increased slightly. Similar patterns were reported earlier for soil nitrification rates (Fierer and Schmel, 2002). Reasons for recovery of some activity are unclear, but the results suggested that both bare and canopy CO-oxidizing communities have similar abilities to adapt to oscillations in water status.

The sensitivity of CO oxidation to water stress is similar to that of other soil processes, such as nitrification and aerobic methane oxidation. For instance, nitrification is inhibited by >85% at water potentials <−3 MPa (Stark and Firestone, 1995). Methane oxidation is strongly inhibited by water potentials from −3 to −4 MPa (Schnell and King, 1996). However, unlike methane oxidation, which does not recover from air-drying (Nesbit and Breitenbeck, 1992), CO oxidation resumes significant activity relatively quickly as documented here (Figures 3 and 4). Nitrifiers also survive severe drought and resume activity within minutes after rehydration (Hastings *et al.*, 2000; Fierer and Schmel, 2002; Steenwerth *et al.*, 2005; Gleeson *et al.*, 2008), although some nitrifiers appear more sensitive to water stress than others (Gleeson *et al.*, 2008).

The similar responses of CO oxidation in bare and canopy sites to desiccation and rehydration seem remarkable considering the differences in water regimes they experience. Water potential at canopy sites remains high, near 0 MPa, even during relatively dry periods (for example, Figure 1), whereas in contrast, bare site cinders experience diurnal variations with water potentials at times falling to values <−60 MPa (for example, Figure 1).

Although bare site water potentials regularly reach extreme values, they also frequently rise to near 0 MPa during periods of rainfall, and at night as temperatures decrease and dew forms. Thus, bare site CO oxidizers experience favourable water potentials for a significant fraction of the day. This may reduce the selective pressure for specific adaptations that would maintain activity during periods of moderate water stress, and account for the similarity in responses observed for the bare and canopy sites.

Alternatively, exposure to water stress may select for stress tolerance by most, if not all, of the initial successful colonists of unvegetated, newly formed volcanic deposits. Over time, changes resulting from plant colonization or other variables may lead to shifts in community composition, but stress tolerance might be retained if new communities are largely drawn from members of the original assemblages. Thus, responses of CO oxidation by canopy and bare sites to imposed water stress would be similar.

Thus, dominance of bare site CO-oxidizing communities by a putative *Firmicutes* clade may reflect water availability, whereas dominance of canopy site CO oxidizers by *Proteobacteria* may reflect plant impacts (Weber and King, submitted). This distribution is consistent with findings of earlier studies, which indicate that Gram-positive bacteria (for example, *Firmicutes*) dominate bacterial communities in arid environments, whereas Gram-negative bacteria dominate wetter, vegetated soils (Chen and Alexander, 1973; Rao and Venkateswarlu, 1983; Busse and Bottomley, 1989; Nesbit and Breitenbeck, 1992; Potts, 1994; Jawad *et al.*, 1998; Nicholson *et al.*, 2000; Nagy *et al.*, 2005; Rainey *et al.*, 2005; Chanal *et al.*, 2006; Vriezen *et al.*, 2007; Clark and Hirsch, 2008).

Although the distribution of specific CO-oxidizing taxa between bare and canopy sites could reflect impacts of the different water regimes, activity data indicate that those regimes do not elicit differential responses measured as CO uptake. Similar results have been reported recently for analyses of microbial activity in a grassland soil that appeared to have dramatically oscillating water regimes (Pesaro *et al.*, 2004). These observations suggest that short-term, frequent oscillations between extreme and moderate states do not select for physiological responses markedly different than those for systems that experience primarily moderate conditions.

Depth profiles for maximum potential CO-oxidation rates at the bare and canopy sites provide support for the notion that exposure to periodic extreme water stresses may not strongly limit the distribution and abundance of active CO oxidizers. The bare site community shows no specific adaptive responses in its activity relative to the canopy site, which does not experience such extremes; bare and canopy site CO uptake depth profiles are also similar (King and Weber, 2008). If water potential regimes limited the distribution and abundance of bare site CO oxidizers, CO oxidation should be higher in the subsurface, for which water potentials remain moderate. What has been consistently observed, however, is that the highest maximum potential CO-oxidation rates occur in the upper 0–1 cm interval (Table 1; see also King and Weber, 2008).

Collectively, these observations show that CO oxidation is a water-sensitive process, but that CO oxidation recovers rapidly after extended periods of desiccation. The results here provide initial insights into the constraints of water potential on CO oxidation in unvegetated volcanic substrates, and show that CO oxidation *in situ* is a dynamic process that likely coincides closely with diurnal oscillations in water status. Striking similarities in CO oxidation responses to water stresses in bare and canopy sites indicate that the metabolic response of active CO-oxidizing communities at the two sites is not differentially adapted to water stress, despite contrasting water regimes. Nonetheless, the ability of at least some CO oxidizers to recover from extended periods of desiccation provides evidence that they can persist in water-stressed environments, and offers insights into factors that contribute to their success as pioneering colonizers of unvegetated volcanic deposits.

Acknowledgements

We gratefully acknowledge support from a National Science Foundation Microbial Observatory Grant (NSF-MCB-0348100).

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Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)