Soil Temperature, Matric Potential, and the Kinetics of Microbial Respiration and Nitrogen Mineralization

Donald R. Zak,* William E. Holmes, Neil W. MacDonald, and Kurt S. Pregitzer

ABSTRACT

Soil temperature and matric potential influence the physiological activity of soil microorganisms. Changes in precipitation and temperature can alter microbial activity in soil, rates of organic matter decomposition, and ecosystem C storage. Our objective was to determine the combined influence of soil temperature and matric potential on the kinetics of microbial respiration and net N mineralization. To accomplish this, we collected surface soil (0–10 cm) from two northern hardwood forests in Michigan and incubated samples at a range of temperatures (5, 10, and 25°C) and matric potentials (−0.01, −0.15, −0.30, −0.90 and −1.85 MPa) that encompass field conditions. Soils were maintained at each temperature–matric potential combination over a 16-wk laboratory incubation, during which we periodically measured the production of CO_2 and inorganic N. First-order kinetic models described the accumulation of CO_2 and inorganic N and accounted for 96 to 99% of the variation in these processes. First-order rate constants (k) for net N mineralization significantly increased with temperature, but the k for microbial respiration did not increase in a consistent manner; it was 0.107 wk^{-1} at 5°C, 0.123 wk^{-1} at 10°C, and 0.101 wk^{-1} at 25°C. Matric potential did not significantly influence k for either process. Substrate pools for microbial respiration and net N mineralization declined between −0.01 and −0.30 MPa, and the decline was greatest at the highest soil temperature; this response produced a significant temperature–matric potential interaction. We conclude that high rates of microbial activity at warm soil temperatures (e.g., 25°C) are limited by the diffusion of substrate to metabolically active cells. This limitation apparently lessens as physiological activity and substrate demand decline at relatively cooler soil temperature (e.g., 5°C).

PREDICTING THE EXTENT to which climate change might alter ecosystem C balance and rates of organic matter decomposition is, in part, contingent on a better understanding of the physiological response of soil microorganisms to altered temperature and precipitation regimes. On a global basis, soil organic matter contains twice as much C as the earth's atmosphere (Schlesinger, 1977; Post et al., 1982; Jenkinson et al., 1991), and the release of CO_2 from this globally important pool is mediated by the physiological activities of soil microorganisms. Organic substrates entering the soil serve as a source of energy for heterotrophic biosynthesis, during which microbial respiration returns a portion of substrate C to the atmosphere as CO_2. Some have hypothesized that warmer global temperatures will enhance microbial activity, rates of organic matter decomposition, and hence, the global flux of CO_2 from soil (Jenkinson et al., 1991; Thornley et al., 1991; Schimel et al., 1994; Kirschbaum, 1995). Warmer soil temperatures and increased rates of decomposition have the potential to transform some terrestrial ecosystems into net sources of CO_2, especially arctic tundra and boreal forests, where large amounts of C reside in soil (Oechel et al., 1993; Goulden et al., 1998). However, such a response would be dampened if warmer temperatures were accompanied by drier soil conditions (i.e., more negative matric potentials).

Microbial metabolism in soil is controlled by substrate availability and by soil temperature and soil matric potential. The mineralization of soil organic matter (C or N) conforms to first-order kinetics and rates can be estimated with knowledge of the substrate pool size and the response of the first-order rate constant (k) to soil temperature (Goncalves and Carlyle, 1994). The temperature dependency of this process is often described as an increase in the first-order rate constant, whereas the substrate pool metabolized is assumed to be unaffected by temperature (Stanford et al., 1973; Campbell et al., 1984). However, the temperature dependence of microbial respiration and net N mineralization appears to involve the access to or metabolism of larger substrate pools as soil temperature increases (Ellert and Bettany, 1992; MacDonald et al., 1995; Zogg et al., 1997). One mechanism to explain this observation is a shift in microbial community composition, such that communities at higher temperatures have the ability to access or metabolize substrates that are not used by members of the microbial community at lower temperatures. Using PLFA analysis to characterize microbial communities (Tunlid and White, 1992), Zogg et al. (1997) incubated soil over a range of temperatures (5, 15, and 25°C) and observed increases in the abundance of Gram-positive bacterial PLFAs and declines in the abundance of Gram-negative bacterial and fungal PLFAs, a change in microbial community composition that paralleled a temperature-dependent increase in the amount of C (i.e., increase in substrate pool size) respired by soil microorganisms. If such a response occurs under field conditions, then understanding changes in substrate use by microbial communities in response to changes in temperature or other environmental factors is important for predicting in situ rates of microbial activity (e.g., respiration and net N mineralization).

Soil-matric potential influences microbial activity by modifying substrate availability. Consequently, matric potential alters rates of organic matter mineralization at warmer temperatures, especially if warmer soil temperatures are accompanied by more negative matric potentials. Rates of microbial processes are generally most rapid near field capacity (−0.01 MPa), and they

D.R. Zak and W.E. Holmes, School of Natural Resources & Environment, Univ. of Michigan, Ann Arbor, MI 48109-1115; N.W. MacDonald, Dep. of Biology, Grand Valley State Univ., Allendale, MI 49401-9403; K.S. Pregitzer, School of Forestry and Wood Products, Michigan Technological Univ., Houghton, MI 49931. Received 13 April 1998. *Corresponding author (drzak@umich.edu).

Published in Soil Sci. Soc. Am. J. 63:575–584 (1999).

Abbreviations: PLFA, phospholipid fatty acid; SE, standard error of the mean; θ, volumetric water content.

Fig. 1. Location of two northern hardwood study sites in Upper and Lower Michigan.

linearly decline as soil matric potential becomes more negative (Linn and Doran, 1984; Paul and Clark, 1996). This decline in microbial activity has been attributed to the decreased diffusion of soluble substrates to microbial cells (Griffin, 1981a), reduced microbial mobility that limits access to substrates (Griffin, 1981b; Killham et al., 1993), the lowering of intracellular water potential, which alters enzyme conformation and inhibits activity (Skujins and McLaren, 1967; Csonka, 1989; Brown, 1990), or a combination of these mechanisms (Stark and Firestone, 1995). Therefore, it is likely that declines in soil matric potential below field capacity (−0.01 MPa) will modify the temperature-dependent increase in substrate pools we have observed for microbial respiration and net N mineralization (sensu MacDonald et al., 1995; Zogg et al., 1997). Although a great deal is understood about the influence of temperature and matric potential on microbial activity, we are unaware of any previous study that has explicitly determined how these environmental factors, in combination, influence the first-order kinetic parameters for microbial respiration and net N mineralization. The primary objective of the present study was to determine how matric potential modifies the influence of soil temperature on substrate pools and first-order rate constants for microbial respiration and net N mineralization. To accomplish our objective, we estimated the kinetic parameters for microbial respiration and net N mineralization for a range of field temperatures and matric potentials in two Lake States Spodosols.

MATERIALS AND METHODS

Study Sites

To determine the influence of soil temperature and water potential on the kinetics of microbial respiration and net N mineralization, we collected surface soil (A and E horizons) from two northern hardwood forests spanning a climate gradient in Upper and Lower Michigan, USA (Fig. 1; Table 1). These forests are dominated by *Acer saccharum* Marsh., and they are floristically, edaphically, and functionally representative of many northern hardwood ecosystems throughout the Upper Lake States region (Zak and Pregitzer, 1990; MacDonald et al., 1991; Pregitzer et al., 1992; Zogg et al., 1996). During the 1987 field season, we located three 30- by 30-m plots in each northern hardwood site. In each plot, we used an OmniData III datalogger (Dataloggers, Inc., Logan, UT) to record soil temperature and matric potential at a depth of 15 cm. Measurements were collected at 5-min intervals and automatically averaged for a 3-h period. Seasonal patterns of mean daily soil temperature and matric potential (1987–1995) within each northern hardwood stand are illustrated in Fig. 2 and 3. These data were used to establish the range of soil temperature and matric potential for which we studied the kinetics of microbial respiration and net N mineralization.

Table 1. Climate, soil, and overstory properties of two northern hardwood forests located in Michigan, USA.

	Site A	Site D
Location and climate		
Latitude	46° 52′ N	43° 40′ N
Longitude	88° 53′ W	86° 09′ W
Elevation, m	380	262
Mean annual precipitation, mm	870	850
Mean annual air temperature, °C	4.2	7.6
N deposition, kg N ha^{-1} yr^{-1}	6.8	11.8
Soil properties†		
pH	4.4	4.3
Silt + clay, %	14.8	12.7
Bulk density, Mg m^{-3}	1.45	1.26
Organic C, g C kg^{-1}	11	22
Microbial C, mg C kg^{-1}	213	328
Microbial N, mg N kg^{-1}	62	92
Net N mineralization, mg N kg^{-1} yr^{-1}	45	66
Taxonomic classification	Alfic and Typic Haplorthods	Typic Haplorthod
Overstory properties		
Overstory age, yr in 1997	90	89
Basal area, m^2 ha^{-1}	32	30
Sugar maple basal area, m^2 ha^{-1}	27	25
Aboveground biomass, Mg ha^{-1}	261	234
Sugar maple biomass, Mg ha^{-1}	228	180

† Properties for a depth of 0 to 10 cm. Data have been summarized from Burton et al. (1996), Zogg et al. (1996), and MacDonald et al. (1991, 1995).

Microbial Respiration and Net Nitrogen Mineralization

In November 1996, we randomly collected 16 surface soil cores within each 30- by 30-m plot. At each sample point, we removed the Oi and Oe horizons by hand and collected mineral soil (A and E horizon) to a depth of 10 cm using a 5.4-cm-diam. core. The 16 cores from each plot were composited in the field, stored on ice, and brought to our laboratory within 24 h of field collection. The field-moist samples were homogenized by hand and air dried at room temperature until they attained a constant mass. Exactly 1 kg of air-dried soil from each plot (three per stand) was composited and homogenized on a stand basis. We used the air-dried, composite samples for all laboratory analyses.

We incubated soil subsamples (14 g dry wt.) from each forest stand under factorial combinations of temperature (5, 10 and 25°C) and matric potential (−0.01, −0.15, −0.30, −0.90 and −1.85 MPa) and measured microbial respiration and net N mineralization (sensu Zak et al., 1993). Prior to the start

of our experiment, we used a ceramic-membrane pressure extractor (Soil Moisture Corp., Santa Barbara, CA) to determine the volumetric H_2O content (θ) of each composite sample at matric potentials of −0.01, −0.15, −0.30, −0.90 and −1.85 MPa (Table 2). We then used deionized H_2O to gravimetrically adjust 60 samples from each site to each of the desired matric potentials. Deionized H_2O was added by misting the soil surface with a hand-held sprayer; soil was initially mixed to insure that H_2O was evenly distributed throughout. After the addition of deionized water, subsamples were sealed within 976-mL Mason jars fitted with rubber septa for gas sampling; we used a pressure transducer to ensure each was sealed airtight. Samples were then placed in laboratory incubators maintained at either 5, 10, or 25°C. At 2-wk intervals, we gravimetrically adjusted the matric potential of each subsample by adding deionized H_2O in the aforementioned manner; however, we did not mix the soil after the incubation began. Opening the incubations to add deionized H_2O also allowed us to replenished any O_2 that had been consumed during the prior incubation period. Over the entire experiment, gravimetric soil water contents varied less than 0.1% at each matric potential.

A total of 20 subsamples from each site were incubated at each of the 15 temperature–matric potential treatment combinations (i.e., 600 samples). This allowed us to harvest two replicate subsamples of each treatment combination and site on 10 sampling dates during a 16-wk laboratory incubation. Subsamples harvested on each sampling date were used to determine net N mineralization. To estimate microbial respiration, we measured the CO_2 concentration of headspace gas for the subsamples incubated for the entire 16-wk period (i.e., harvested after 16 wks of incubation). We chose to measure net N mineralization using a sequential harvest, because repeatedly extracting a single subsample would not allow us to maintain a constant matric potential during the incubation. We harvested and extracted 60 subsamples (15 treatment combinations × 2 sites × 2 replicates) at the start of our experiment, after Weeks 1 and 2, and at 2-wk intervals thereafter (i.e., 10 sampling dates) to measure net N mineralization. Ammonium–N and NO_3^-–N were extracted using 28 mL of 2 mol L^{-1} KCl. Each sample was shaken for 30 min on an orbital shaker and then passed through a glass fiber filter (Gelman GF-A, Gelman Corp., Ann Arbor, MI). The filtrate was colorimetrically analyzed for NH_4^+–N and NO_3^-–N using an Alpkem RFA Rapid Flow Analyzer (Alpkem Corp., Clackamas, OR). Net N mineralization was calculated as the increase in NH_4^+–N + NO_3^-–N for the incubation period.

At Weeks 1, 2, and at 2-wk intervals thereafter, we measured the CO_2 concentration of headspace gas in the set of samples incubated for the entire 16 wks; i.e., those destructively harvested at wk 16. Samples of headspace gas (400 μL) were analyzed for CO_2 using a Tracor 540 gas chromatograph (Tracor Instruments, Austin, TX) equipped with a Porapak Q column (Millipore Corp., Milford, MA) and a thermal conductivity detector. The column was maintained at 55°C during the analysis and He (25 mL min^{-1}) was used as a carrier gas. Carbon dioxide (CO_2–C) and inorganic N production were expressed on a mass basis (mg kg^{-1}) for each individual soil incubation.

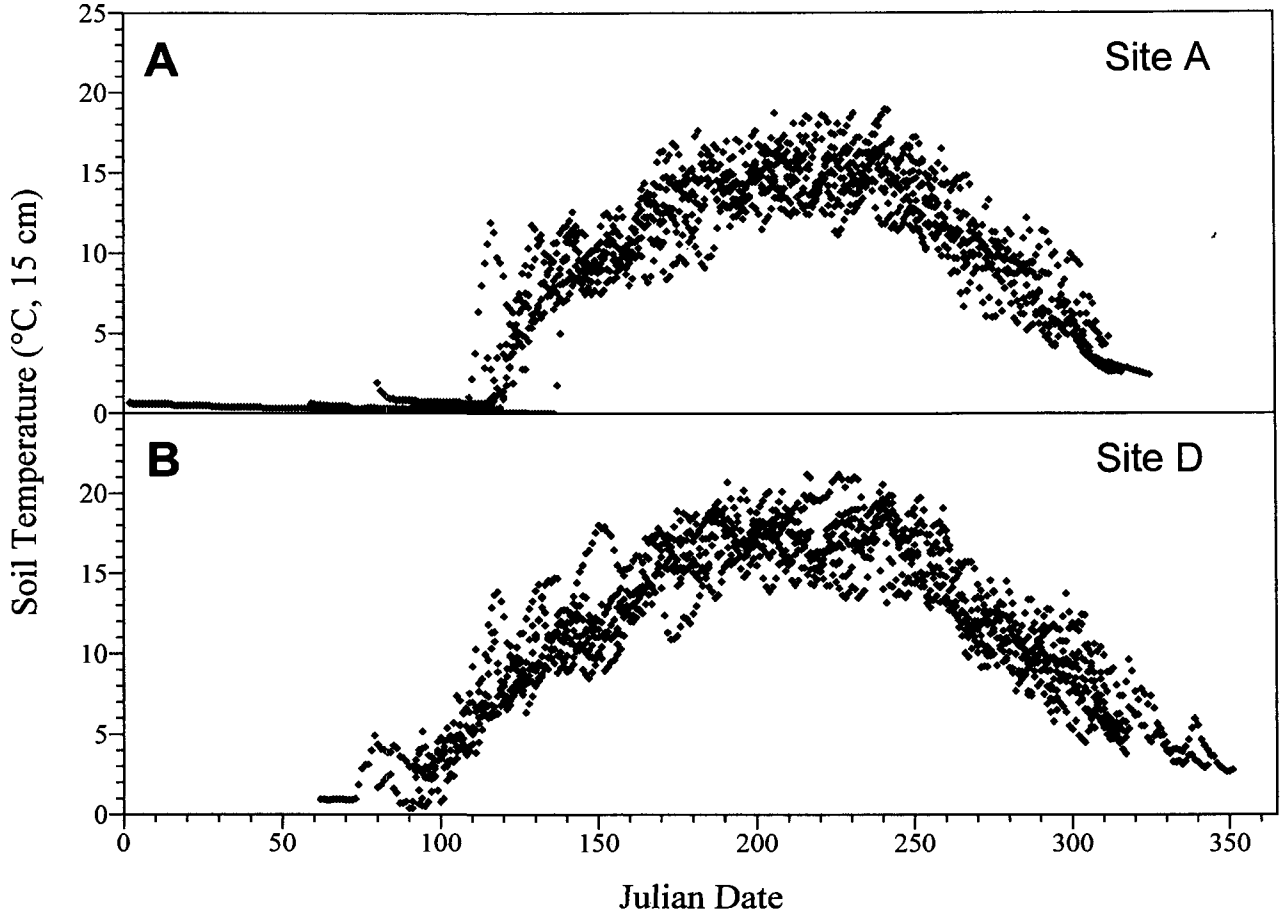

Fig. 2. Mean daily soil temperatures (15 cm depth) for two northern hardwood forests located in Upper and Lower Michigan. Data points are daily means from 1987 to 1995.

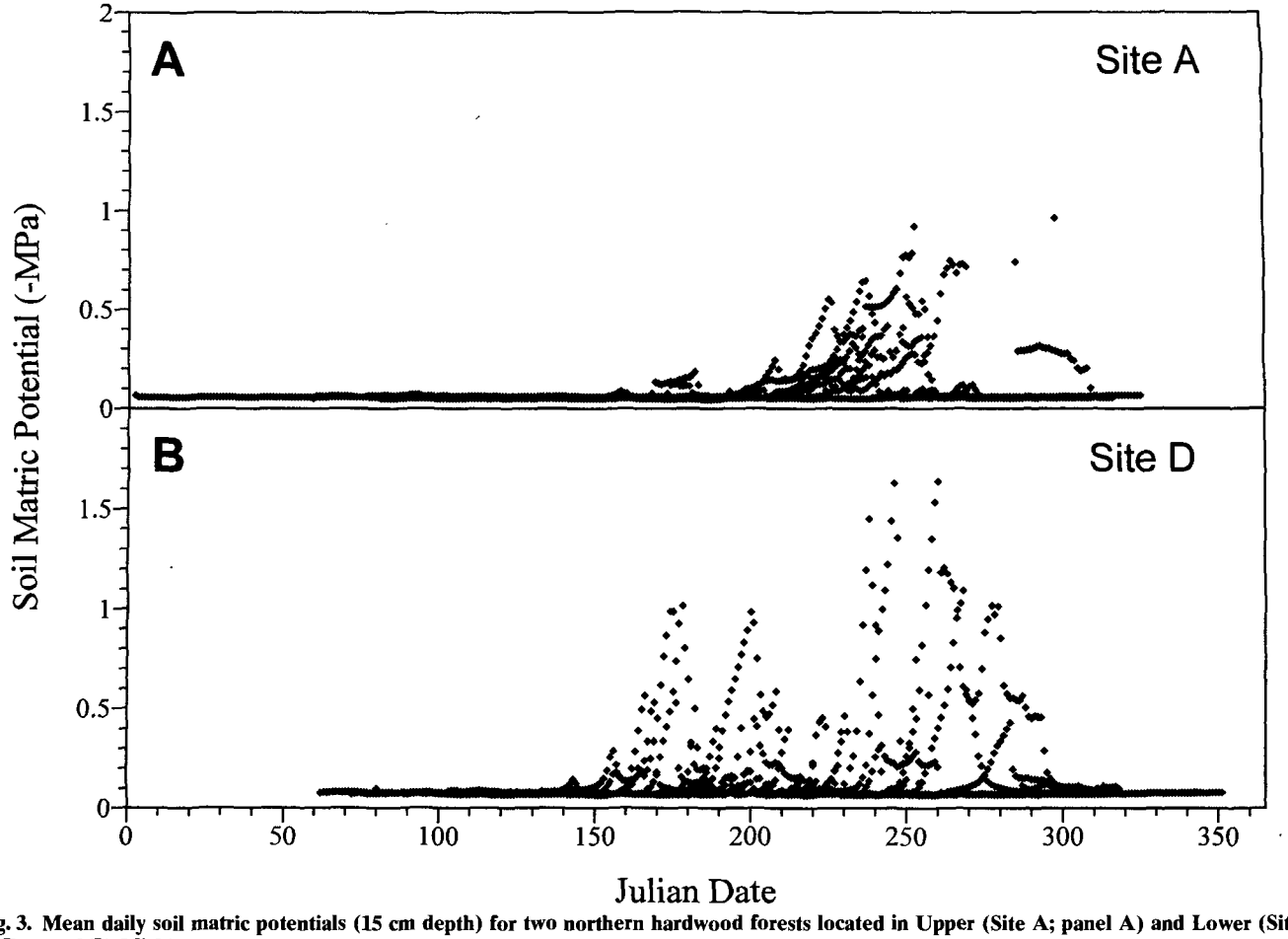

Fig. 3. Mean daily soil matric potentials (15 cm depth) for two northern hardwood forests located in Upper (Site A; panel A) and Lower (Site D; panel B) Michigan.

Statistical Analyses

We constructed product accumulation curves for microbial respiration by summing the amount of CO_2–C produced in the 16-wk incubation. Product accumulation curves for inorganic N were constructed by averaging the amount of NH_4^+–N + NO_3^-–N in replicate samples harvested on each sampling date (i.e., two from each site–temperature–matric potential treatment combination). Using nonlinear least squares regression, we fit a first-order rate equation to each product accumulation curve:

$$Y = A_o(1 - e^{-kt}) \quad [1]$$

where, Y is the cumulative CO_2–C or inorganic N (mg C or N kg^{-1}) produced at time t, A_o is an estimate of the substrate pool present at the start of the experiment (mg C or N kg^{-1}), and k is the first-order rate constant (wk^{-1}).

We compared estimates of A_o and k using an ANOVA for a nested design with two factorial treatments. In our analysis, temperature (n = 3) and matric potential (n = 5) treatments are crossed, and these treatment combinations were nested within sites (n = 2). Main effect and interaction means were compared using a Fisher's protected least significant difference procedure. We also explored the relationship between kinetic parameters and θ using linear regression; significance for all statistical analyses was accepted at α = 0.05.

Table 2. Matric potentials and gravimetric water contents of two sandy Spodosols from northern Michigan. The matric potentials listed below represent the range of values measured in the field from 1987 to 1995.

	Matric potential ψ, mPa				
	−0.01	−0.15	−0.30	−0.90	−1.85
Volumetric water content (θ mL cm^{-3})					
Site A	0.330	0.184	0.149	0.107	0.087
Site D	0.215	0.113	0.097	0.076	0.063

RESULTS

Microbial Respiration

The first-order model was highly significant and accounted for 98 to 99% of the variation in CO_2–C accumulation during the 16-wk incubation. In Fig. 4, we illustrate the response of microbial respiration to the soil temperature and matric potential using the derived first-order kinetic parameters. Substrate pool estimates for microbial respiration (i.e., respired C) were significantly influenced by a temperature–matric potential interaction, wherein a decline in matric potential from −0.01 to −0.30 MPa resulted in a significant and substantially larger decrease in respired C at 25°C than at either of the lower temperatures (Fig. 5A; Table 3). There was a significant linear relationship between respired C pools and mean θ at 25°C (r^2 = 0.90); this relationship was significant but weaker at 10°C (r^2 = 0.66); and it was not significant at 5°C.

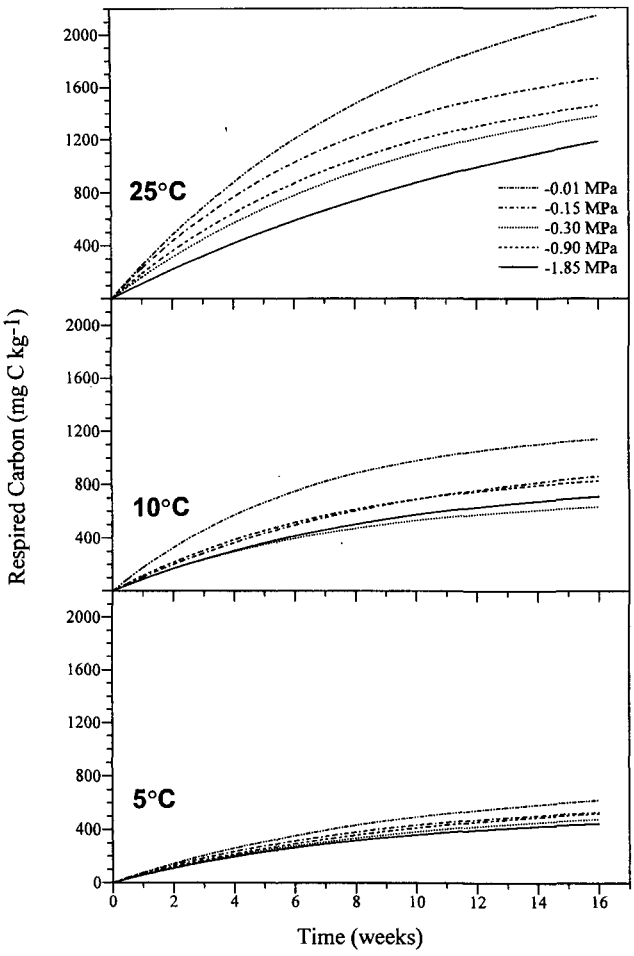

Fig. 4. The production of respired C at different soil temperatures and matric potentials was estimated with the use of the kinetic parameters for microbial respiration. The pool of respired C and k_{resp} was determined at each temperature and matric potential with the use of 16-wk laboratory incubations. Mean (see Table 3) of kinetic parameters for each temperature–matric potential treatment combination were used to generate the production of respired C.

Temperature and matric potential were significant main effects in the ANOVA model and substantially influenced the amount of C respired during the 16-wk incubation. However, temperature had a relatively greater influence on respired C pools than did soil matric potential. The mean pool of C respired at 25°C (1987 ± 30.5 mg C kg^{-1}; mean ± SE) was significantly greater than pools respired at 10°C (966 ± 70.3 mg C kg^{-1}) or at 5°C (638 ± 100.9 mg C kg^{-1}), indicating a temperature-dependent increase in pool size. The mean respired C pool at −0.01 MPa was 1582 ± 266.6 mg C kg^{-1}, and it was significantly greater than the mean pool size at the other matric potentials (range: 1199 mg C kg^{-1} at −0.15 MPa to 1086 mg C kg^{-1} at −1.85 MPa). Averaged across temperature–matric potential treatment combinations, the mean pool of respired C at Site D (1305 ± 132.1 mg C kg^{-1}) was significantly greater than that at Site A (1089 ± 106.8 mg C kg^{-1}), a pattern that paralleled differences in soil organic C content (Table 1). Site did not significantly interact with either temperature or matric potential.

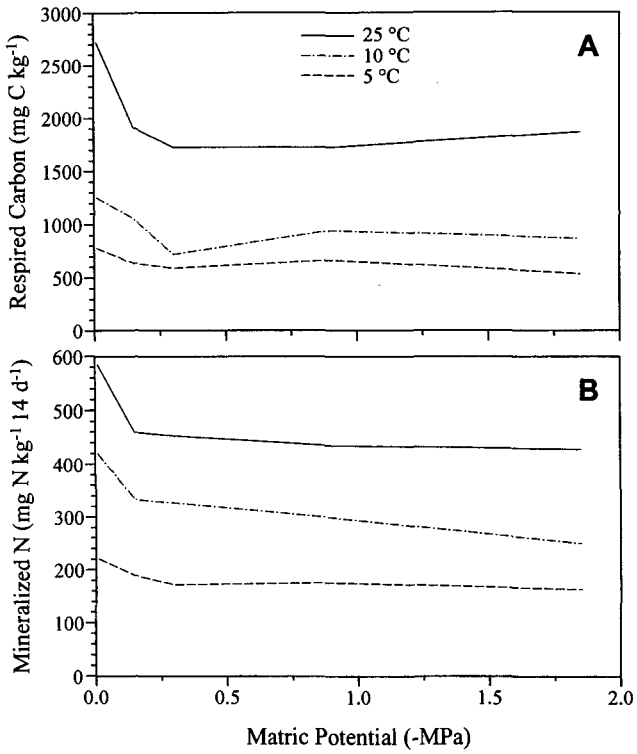

Fig. 5. The interaction of temperature and matric potential on the pool of respired C (A) and the amount of N mineralized after 2 wk of laboratory incubation (B). The influence of matric potential on reducing the pools of respired C or amounts of mineralized N was much greater at 25°C than at either 10°C or 5°C; those differences were significant. Values are interaction means (averaged across sites).

Rates constants for microbial respiration (k_{resp}) were not significantly influenced by matric potential, but they did differ significantly among temperature treatments (Table 3). Although the influence of temperature was significant, k_{resp} did not increase in a consistent manner with temperature. For example, mean k_{resp} at 5°C was 0.107 wk^{-1}; this value increased to 0.123 wk^{-1} at 10°C and then declined to 0.101 wk^{-1} at 25°C. We also observed a significant difference in k_{resp} between the two sites, but these differences were relatively small. For example, the mean k_{resp} was 0.120 ± 0.0063 wk^{-1} in Site D soil and 0.103 ± 0.0059 wk^{-1} in Site A soil. The turnover times ($1/k_{resp}$) derived from these values ranged from 9 to 10 wk. Despite significant differences in k_{resp} among these forest soils, the narrow range of values and short turnover times indicate that substrates for microbial respiration were similar and relatively labile. The site × temperature and site × matric potential interactions were not significant, nor was the three-way interaction among these variables.

Net Nitrogen Mineralization

Net N mineralization could also be well described by the first-order kinetic model, which accounted for 96 to 99% of the variation in inorganic N accumulation over the 16-wk incubation. The response of net N mineralization to our temperature–matric potential combinations is depicted in Fig. 6, which was constructed using the

Table 3. The response of first-order kinetic parameters to soil temperature and matric potential. Values are means (averaged across sites) of the temperature–matric potential treatment combinations (n = 4) with their standard errors listed in parentheses.

Temprature	Matric potential	Respired C	k_{resp}	Mineralized N	k_{min}
°C	MPa	mg C kg^{-1}	wk^{-1}	mg N kg^{-1}	wk^{-1}
5	−0.01	775	0.102	413	0.041
		(110.4)	(0.0060)	(41.41)	(0.0005)
	−0.15	635	0.115	308	0.075
		(66.8)	(0.0142)	(41.7)	(0.0107)
	−0.30	586	0.107	288	0.083
		(26.5)	(0.0152)	(19.3)	(0.0113)
	−0.90	662	0.098	272	0.089
		(5.8)	(0.0026)	(30.6)	(0.0068)
	−1.85	533	0.115	237	0.108
		(32.3)	(0.0088)	(19.6)	(0.0143)
10	−0.01	1252	0.152	318	0.138
		(176.3)	(0.0062)	(18.6)	(0.0105)
	−0.15	1054	0.106	436	0.089
		(78.4)	(0.0268)	(64.3)	(0.0200)
	−0.30	718	0.134	399	0.101
		(212.3)	(0.0159)	(44.2)	(0.0100)
	−0.90	942	0.132	379	0.106
		(99.3)	(0.0195)	(33.0)	(0.0102)
	−1.85	864	0.109	393	0.076
		(102.7)	(0.0150)	(34.4)	(0.0107)
25	−0.01	2720	0.097	555	0.117
		(222.6)	(0.0157)	(63.8)	(0.0078)
	−0.15	1910	0.128	488	0.132
		(97.5)	(0.0218)	(99.2)	(0.0340)
	−0.30	1724	0.100	548	0.128
		(134.0)	(0.0126)	(80.5)	(0.0148)
	−0.90	1722	0.117	524	0.145
		(54.3)	(0.0111)	(72.7)	(0.0124)
	−1.85	1863	0.063	505	0.108
		(99.7)	(0.0173)	(59.0)	(0.0056)
Fisher's LSD		367.1	ns	38.8	0.0180

derived first-order kinetic parameters. There was a significant interaction of temperature and matric potential on mineralized N pools, arising from a decline in mineralized N between −0.01 and −0.15 MPA at 5°C and an increase in mineralized N between these same matric potentials at 10°C (Fig. 6; Table 3). Although this interaction was significant, mineralized N pools estimated by the first-order equation did not respond to temperature and matric potential in a predictable manner. Much of this variability undoubtedly results from our sequential harvest of samples to construct product accumulation curves, which probably influenced the estimation of first-order kinetic parameters. To reduce this variability, we compared the amount of N mineralized after 2 wk of incubation and found a significant temperature–matric potential interaction that was similar to that relationship for respired C (Fig. 5B).

Mineralized N responded to matric potential to a lesser degree than respired C pools did (compare Fig. 4 and 6). At each incubation temperature, mineralized N pools changed very little at matric potentials more negative than −0.30 MPa (Table 3). The main effect of matric potential (averaged across temperature and site) on mineralized N was not significant, but mineralized N declined from 429 mg N kg^{-1} at −0.01 MPa to 412 mg N kg^{-1} at −0.30 MPa and then changed little between −0.30 and −1.85 MPa. However, the mean mineralized N pool differed significantly among incubation temperatures. Mineralized N was 303 ± 18.7 mg N kg^{-1}

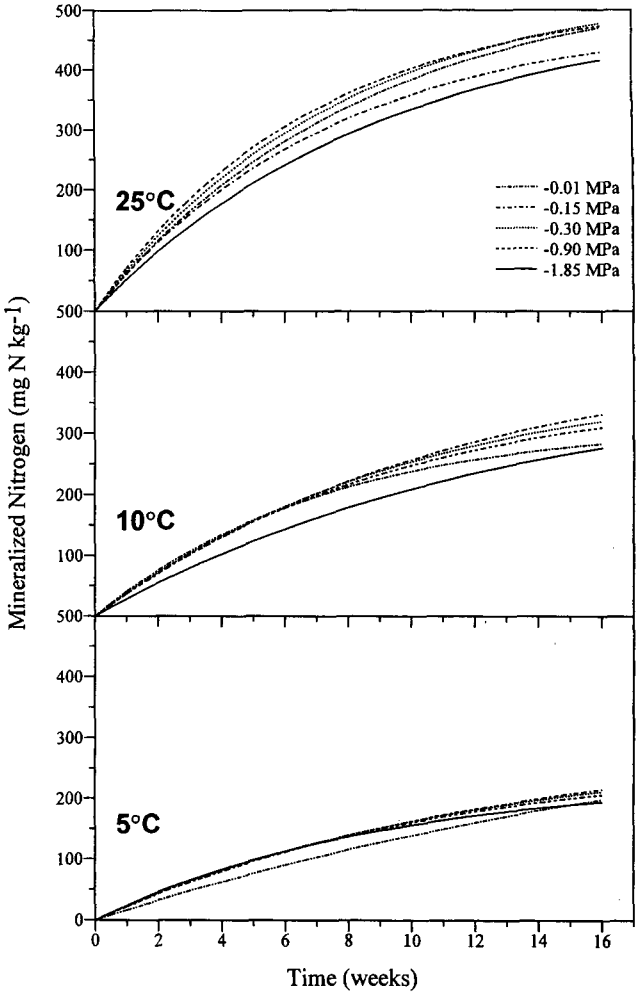

Fig. 6. The production of mineralized N at different soil temperatures and matric potentials was estimated with the use of the first-order kinetic parameters for net N mineralization. The pools of mineralized N and k_{min} were determined at each temperature and matric potential with the use of 16-wk laboratory incubations. Mean (see Table 3) kinetic parameters for each temperature–matric potential treatment combination were used to generate the production of mineralized N.

at 5°C, 384 ± 18.8 mg N kg^{-1} at 15°C, and 524 ± 61.3 mg N kg^{-1} at 25°C (LSD = 33.1 mg N kg^{-1}). The mean mineralized N pool was 479 ± 26.1 mg N kg^{-1} in Site D soil, and it was significantly greater than the mean at Site A (329 ± 15.1 mg N kg^{-1}) where soil C content was lower (Table 1). The interaction of site with either temperature or matric potential was not significant.

First-order rate constants for net N mineralization (k_{min}) were significantly influenced by the interaction of temperature and matric potential (Table 3). Between matric potentials of −0.01 and −0.15 MPa, k_{min} declined from 0.138 wk^{-1} to 0.089 wk^{-1} at 15°C and increased from 0.041 wk^{-1} to 0.075 wk^{-1} at 5°C (Table 3); these differences were significant. At each soil temperature, matric potential did not have significant influence on k_{min} at values more negative than −0.3 MPa (Table 3). Rate constants for net N mineralization were not significantly influenced by matric potential (main effect), but they did differ significantly among incubation tempera-

tures. Rate constants increased consistently with increasing temperature from 0.079 ± 0.006 wk^{-1} at 5°C, 0.102 ± 0.007 wk^{-1} at 10°C, to 0.126 ± 0.008 wk^{-1} at 25°C. Consequently, turnover of mineralized N pools ($1/k$) ranged from 12.6 wk at 5°C to 7.9 wk at 25°C, a relatively narrow range of values. Rate constants for net N mineralization did not significantly differ among sites, suggesting that substrate chemistry was similar among these floristically similar northern hardwood forests. We also observed no interactions among site and temperature or matric potential.

DISCUSSION

The physiological response of soil microorganisms to soil temperature and matric potential will mediate the extent to which climatic change alters the flux of CO_2 and inorganic N from soil organic matter. Although many studies have focused on how warmer soil temperatures will alter the decomposition of soil organic matter, few have experimentally derived the relationship between soil temperature, matric potential, and the kinetic parameters for microbial respiration and net N mineralization. Further study of this relationship is important, because many models of soil organic matter decomposition are based on the assumption of a temperature-dependent first-order rate constant(s) and temperature independent substrate pool(s) (Parton et al., 1987; Jenkinson, 1990). We previously reported that substrate pools for microbial respiration and net N mineralization that were estimated by the first-order equation were larger at warmer soil temperatures, but such a response does not support the aforementioned assumption of temperature dependence (MacDonald et al., 1995; Zogg et al., 1997). Because soil matric potential can limit substrate availability for microbial metabolism (Griffin, 1981a, 1981b), we reasoned matric potential could modify the kinetics of microbial respiration and net N mineralization. In our experiment respired C and mineralized N pools declined as matric potential decreased from −0.01 MPa to −0.30 MPa, and this reduction was greatest at the warmest soil temperature (i.e., 25°C). It appears that high rates of microbial activity at relatively warm soil temperatures are limited by the diffusion of substrate to metabolically active cells and that this limitation lessens as physiological activity and substrate demand decline at relatively cooler soil temperatures (i.e., 5°C).

In the northern hardwood forests that we studied, soil temperature and matric potential exhibit marked seasonal variation (Fig. 2 and 3), which our laboratory treatments were designed to encompass. Over this range of soil temperatures and matric potentials, we found that soil temperature had a greater influence on microbial activity than matric potential. For example, mean respired C increased 300% from 5 to 25°C, but it increased only 40% from −1.85 to −0.01 MPa. Similarly, we observed a 70% increase in mineralized N pools from 5 to 25°C and a 10% increase from −1.85 to −0.01 MPa. Temperature also significantly increased k_{min} for net N mineralization, although k_{resp} did not consistently increase with temperature. In contrast, soil matric potential had no effect on k for either microbial respiration or net N mineralization. Although temperature and matric potential differed in their influence, they should not be considered independently when describing microbial activity in soil (sensu Cassman and Munns, 1980).

The interaction between soil temperature and matric potential is likely important in controlling the flux of CO_2 from soil organic matter under field conditions. Support for this contention comes from interpreting our laboratory incubation study in the context of field soil temperature and matric potentials. We observed that the greatest change in respired C pools occurred between −0.01 and −0.30 MPa, and the greatest degree of change was at the warmest soil temperature (25°C). Between −0.30 and −1.85 MPa, we observed very little change in respired C pools at each respective temperature (see Fig. 5A). Throughout our 8-year climate record, daily soil matric potentials more negative than −0.30 MPa occurred an average of 10 d yr^{-1} at Site A and 18 d yr^{-1} at Site D. Thus, over most of the year, in situ matric potentials lie within the range over for which we observed the greatest effects on respired C pools. Moreover, fluctuations in matric potential between −0.01 to −0.30 MPa should be most important during mid-summer when soil temperatures commonly vary from 15 to 25°C (Fig. 2), the range of temperature for which soil matric potential exerted the greatest influence on microbial respiration.

The relative contribution of plant roots and soil microorganisms to the flux of CO_2 from soil can change as a function of soil matric potential, especially during mid-summer when soil temperatures are relatively warm and drying conditions occur. Recently, Burton et al. (1998) studied the influence of soil matric potential on fine root respiration in sugar maple-dominated northern hardwood forests, including the same sites used in the present study. They found a significant linear decline (2.2-fold at 25°C) in fine root respiration between −0.01 and −0.50 MPa, which differs from the range of response we observed for microbial respiration. We found that the pool of C respired by soil microorganisms decreased significantly from −0.01 to −0.30 MPa, but it changed little at more negative matric potentials. Thus, as soil dries during summer, fluctuations in soil matric potential between −0.30 and −0.50 MPa can alter the relative contribution of fine roots and soil microorganisms to the flux of CO_2 from soil. For example, microbial respiration would change little as matric potential declined from −0.30 to −0.50 MPa, but fine root respiration would continue to decline. The extent of such an effect on soil respiration cannot be understood from our present results. Given the infrequent periods during which matric potential drops below −0.30 MPa in our study sites, such a response is not likely to significantly influence the annual flux of CO_2 from soil. Nonetheless, this may be important in other ecosystems in which soils experience more frequent drying conditions.

Although we did not observe a consistent pattern in mineralized N pools across our temperature–matric potential treatments, we did observe a significant inter-

action of temperature and matric potential on the amount N mineralized over the first 2 wk of incubation. We chose to evaluate our data in this manner to reduce variability associated with our sequential harvest and to compare our results with others in the literature. Declines in net N mineralization between −0.01 and −0.30 MPa were greatest at 25°C and progressively lessened at the lower soil temperatures (Fig. 5B). Cassman and Munns (1980) observed a similar decline in net N mineralization ($\mu g\ N\ g^{-1}\ 14\ d^{-1}$) between −0.01 and −0.20 MPa and very little change as matric potentials dropped below −0.20 MPa. They also observed a significant interaction between soil temperature and matric potential in which the decline in net N mineralization rate from −0.01 to −0.20 MPa was greatest at the warmest soil temperatures (30°C). Our results and those of Cassman and Munns (1980) demonstrate an interactive effect of temperature and matric potential on microbial activity, but others have found very little evidence for an interactive effect (Greaves and Carter, 1920; Robinson, 1957; Miller and Johnson, 1964; Reichman et al., 1966; Stanford and Epstein, 1974; Kladivko and Keeney, 1987).

There are theoretical reasons why one might expect temperature and matric potential to influence microbial respiration and net N mineralization in a multiplicative manner, thus producing a statistically significant interaction. Diffusion controls the flow of organic substrates to microbial cells and the flow of extracellular enzymes to insoluble substrates, the rates of which are influenced by soil temperature and matric potential (Hillel, 1980). Equation [2] describes diffusion rate (J_D) as the product of the diffusion coefficient for a particular ion (D_o), volumetric

$$J_d = -D_o\theta^2\xi \frac{\partial c}{\partial x} \qquad [2]$$

water content (θ) of soil, tortuosity (ξ) of flow in soil, and the concentration gradient ($\partial c/\partial x$) of the diffusing ion (Hillel, 1980). In Eq. [2], ξ is defined as the ratio of the straight-line path for diffusion and the actual roundabout path taken by an ion in soil; as such, ξ declines as the path for diffusion lengthens (i.e., *actual* tortuosity increases). In turn, ξ is a function of soil water content, because the film of water surrounding soil particles becomes thinner as θ declines; thus, the path length for diffusion increases and the value of ξ becomes smaller (as matric potential becomes more negative). Temperature influences J_D because D_o increases linearly with absolute temperature (Weast, 1978). As such, J_D should exhibit a exponential decline with θ, the rate of which will increase with temperature (i.e., more rapid decline at warmer temperatures). This is similar to the pattern we observed for respired C pools and net N mineralization rates (Fig. 5A and 5B). Given the components of Eq. [2] and their mathematical relationship, temperature and soil matric potential influence diffusion in a multiplicative manner and, thus, have the potential to similarly influence substrate availability.

Although changes in diffusion may offer a physical explanation for the significant temperature–matric potential interaction we observed, patterns of microbial activity among our temperature–matric potential treatments cannot be fully explained by this process. Clearly, temperature influences physiological activity and, consequently, the demand for substrate. It appears that microbial activity is limited by diffusion at warmer soil temperatures where high rates of physiological activity create a large demand for substrate. Substrate diffusion is likely to be less limiting at lower temperatures (e.g., 5°C) due to reduced physiological activity and low substrate demand. Thus, matric potential, through its influence on substrate diffusion, can become an increasingly important constraint on microbial activity as soil temperatures increase from 5 to 25°C. This response would thus be influenced by both the physical movement of substrate to microbial cells and their physiological demand for substrate. Stark and Firestone (1995) observed that substrate diffusion limited rates of nitrification between −0.01 and −0.60 MPa in a xeric oak woodland–annual grassland soil incubated at 23°C, a somewhat broader range of influence than we observed in our mesic sandy soils.

Theoretically, diffusion can influence the *rate* at which substrate moves to metabolically active microbial cells. However, it is unlikely that diffusion controls substrate pool *size*, unless discontinuities in the film of water surrounding soil particles isolate substrates and prevent them from diffusing to metabolically active soil microorganisms. Thus, one would expect diffusion to largely alter k but have a minor influence of substrate pool size. This expectation is not consistent with our data, in which we observe a significant decline in substrate pool size as matric potentials dropped from −0.01 to −0.30 MPa. Two mechanisms can account for changes in substrate pools with decreasing matric potential. First, as matric potential declines, the concentration of ions in soil solution increases, which causes soil microorganisms to accumulate ions within their cells to maintain an osmotic balance (Csonka, 1989). High intracellular ion concentrations can alter enzyme conformation and reduce activity (Skujins and McLaren, 1967; Csonka, 1989). Stark and Firestone (1995) found such an effect at matric potentials more negative than −0.60 MPa. If enzyme activity were reduced by osmotic regulation in our experiment, then changes in enzyme activity could potentially alter substrate use. Alternatively, different groups of soil microorganisms may be active under different matric potentials. For example, soil bacteria and fungi differ widely in their ability to withstand water stress. Fungi are able to remain metabolically active at water potentials that inhibit bacterial metabolism (Paul and Clark, 1996). If our matric potential treatments altered the activity of different microbial groups, then changes in fungal and bacterial metabolism could have given rise to the use of different substrate pools as matric potential declined from −0.01 to −0.30 MPa. Our experiment was not designed to address these alternatives and further experimentation would be needed to determine their importance.

In summary, soil temperature and matric potential

were both important in modifying the kinetics of microbial respiration and net N mineralization. Rate constants for these processes changed with soil temperature, but were not influenced by matric potential. In contrast, soil temperature and matric potential interacted in a significant manner to influence substrate pools for microbial respiration and net N mineralization. From this analysis, we conclude that high rates of microbial activity at relatively warm soil temperatures (i.e., 25°C) are limited by the diffusion of substrate to metabolically active cells. This limitation apparently lessens as physiological activity and substrate demand decline at lower soil temperatures (e.g., at 5°C). Because our laboratory treatments encompass field temperatures and matric potentials, it is likely that the kinetic responses we observed would also occur in the nature. We conclude that soil temperature and matric potential interact to influence substrate diffusion and physiological activity under field conditions. Simple assumptions of a single pool of labile substrate and a temperature-dependent first-order rate constant appear to be unfounded.

ACKNOWLEDGMENTS

We thank Diana Randlett and Greg Zogg for their assistance in the field, in the laboratory, and with data analyses. Linda Abriola and Lee Glascoe provided invaluable advice on substrate diffusion in soil. This study was supported by grants from the National Science Foundation (DEB 94-96197 and DEB 96-29842), the Environmental Protection Agency (EPA-C-R824979-01), and the USDA Forest Service Northern Global Change Program.

REFERENCES

Brown, A.D. 1990. Microbial water stress physiology. Wiley & Sons, Ltd., Chichester, UK.

Burton, A.J., K.S. Pregitzer, G.P. Zogg, and D.R. Zak. 1996. Latitudinal variation in sugar maple fine root respiration. Can. J. For. Res. 21:1761–1768.

Burton, A.J., K.S. Pregitzer, G.P. Zogg, and D.R. Zak. 1998. Drought reduces root respiration in sugar maple forests. Ecol. Applic. 8:771–778.

Cassman, K.G., and D.N. Munns. 1980. Nitrogen mineralization as affected by soil moisture, temperature and depth. Soil Sci. Soc. Am. J. 44:1233–1237.

Campbell, C.A., Y.W. Jame, and G.E. Winkleman. 1984. Mineralization rate constants and their use for estimating nitrogen mineralization in some Canadian prairie soils. Can. J. Soil Sci. 64:333–343.

Csonka, L.N. 1989. Physiological and genetic responses of bacteria to osomotic stress. Microbiol. Rev. 52:121–147.

Ellert, B.H., and J.R. Bettany. 1992. Comparison of kinetic models for describing net sulfur and nitrogen mineralization. Soil Sci. Soc. Am. J. 56:1133–1141.

Goulden, M.L., S.C. Wofsy, J.W. Harden, S.E. Trumbore, P.M. Crill, S.T. Gower, T. Fries, B.C. Daube, S.-M. Fan, S.J. Sutton, A. Bazzaz, and J.W. Munger. 1998. Sensitivity of boreal forest carbon balance to soil thaw. Science 279:214–217.

Goncalves, J.L.M., and J.C. Carlyle. 1994. Modeling the influence of moisture and temperature on net nitrogen mineralization in a forested sandy soil. Soil Biol. Biochem. 26:1557–1564.

Greaves, J.E., and E.G. Carter. 1920. Influence of moisture on the bacterial activity of soil. Soil Sci. 10:361–398.

Griffin, D.M. 1981a. Water potential as a selective factor in the microbial ecology of soils. p. 141–151. *In* L.F. Elliot et al. (ed.) Water potential relations in soil microbiology. SSSA Special Publication 9. SSSA, Madison, WI.

Griffin, D.M. 1981b. Water and microbial stress. p. 91–136. *In* M. Alexander (ed.) Advances in microbial ecology. Vol. 5. Plenum Publ., New York.

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

Jenkinson, D.S. 1990. The turnover of organic carbon and nitrogen in soil. Phil. Trans. R. Soc. London B329:361–368.

Jenkinson, D.S., D.E. Adams, and A. Wild. 1991. Model estimates of CO_2 emissions from soil in response to global warming. Nature (London) 351:304–306.

Killham, K., A. Amato, and J.N. Ladd. 1993. Effect of substrate location in soil and soil pore-water regime on carbon turnover. Soil Biol. Biochem. 25:57–62.

Kirschbaum, M.U.F. 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biol. Biochem. 27:753–760.

Kladviko, E.J., and D.R. Keeney. 1987. Soil nitrogen mineralization as affected by water and temperature interactions. Biol. Fertil. Soils 5:248–252.

Linn, D.M., and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48:1267–1272.

MacDonald, N.W., A.J. Burton, M.F. Jurgensen, J.W. McLaughlin, and G.D. Mroz. 1991. Variation in forest soil properties along a Great Lakes air pollution gradient. Soil Sci. Soc. Am. J. 55: 1709–1715.

MacDonald, N.W., D.R. Zak, and K.S. Pregitzer. 1995. Temperature effects on the kinetics of microbial respiration and the net mineralization of N and S. Soil Sci. Soc. Am. J. 59:233–240.

Miller, R.D., and D.D. Johnson. 1964. The effect of soil moisture tension on carbon dioxide evolution, nitrification, and nitrogen mineralization. Soil Sci. Soc. Am. Proc. 28:644–647.

Oechel, W.C., S. Cowles, N. Grulke, S.J. Hastings, B. Lawerence, T. Prudhomme, G. Reichers, B. Strain, D. Tissue, and G. Vourlitis. 1993. Transient nature of CO_2 fertilization in arctic tundra. Nature (London) 371:500–503.

Parton, W.J., D.S. Schimel, C.V. Cole, and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Sci. Soc. Am. J. 51:1173–1179.

Paul, E.A., and F.E. Clark. 1996. Soil Microbiology and Biochemistry. 2nd ed. Academic Press, New York.

Post, W.M. W.R. Emanuel, P. Zinke, and A.G. Strangerberger. 1982. Soil carbon pools and world life zones. Nature 298:156–159.

Pregitzer, K.S., A.J. Burton, G.D. Mroz, H.O. Liechthy, and N.W. MacDonald. 1992. Foliar sulfur and nitrogen along an 800-km pollution gradient. Can. J. For. Res. 22:1761–1769.

Reichmann, G.A., D.L. Grunes, and F.G. Viets, Jr. 1966. Effect of soil moisture on ammonification and nitrification in two northern plains soils. Soil Sci. Soc. Am. Proc. 30:363–366.

Robinson, J.B.D. 1957. The critical relationship between soil moisture content and the region of wilting point and the mineralization of natural soil nitrogen. J. Agric. Sci. (Cambridge) 49:100–105.

Schimel, D.S., B.H. Braswell, E.A. Holland, R. McKeown, D.S. Ojima, T.H. Painter, W.J. Parton, and A.R. Townsend. 1994. Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. Global Biogeochem. Cycles 8:279–293.

Schlesinger, W.H. 1977. Carbon balance in terrestrial detritus. Annu. Rev. Ecol. Syst. 8:51–81.

Skujins, J.J., and A.D. McLaren. 1967. Enzyme reaction rates at limited water activities. Science 158:1569–1570.

Stanford, G., M.H. Frere, and D.H. Schwaninger. 1973. Temperature coefficient of soil nitrogen mineralization. Soil Sci. 115:321–323.

Stanford, G., and E. Epstein. 1974. Nitrogen mineralization-water relations in soils. Soil Sci. Soc. Am. Proc. 38:103–106.

Stark, J.M., and M.K. Firestone. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Appl. Environ. Microbiol. 61:28–221.

Thornley, J.H.M., D. Fowler, and M.G.R. Cannel. 1991. Terrestrial carbon storage resulting from CO_2 and nitrogen fertilization in temperate grasslands. Plant Cell Environ. 14:1007–1011.

Tunlid, A., and D.C. White. 1992. Biochemical analysis of biomass, community structure, nutrient status, and metabolic activity of microbial communities in soil. p. 229–262. *In* J. Bollag and G. Stotsky (ed.) Soil biochemistry. Marcel Dekker, New York.

Weast, R.C. 1978. CRC handbook of chemistry and physics. 58th ed. CRC Press, West Palm Beach, FL.
Zak, D.R., and K.S. Pregitzer. 1990. Spatial and temporal variability of nitrogen cycling in northern Lower Michigan. For. Sci. 36:367–380.
Zak, D.R., D.F. Grigal, and L.F. Ohmann. 1993. Kinetics of microbial respiration and nitrogen mineralization in Great Lakes Forests. Soil Sci. Soc. Am. J. 57:1100–1106.
Zogg, G.P., D.R. Zak, A.J. Burton, and K.S. Pregitzer. 1996. Fine root respiration in northern hardwood forests in relation to temperature and nitrogen availability. Tree Physiol. 16:719–725.
Zogg, G.P., D.R. Zak, D.B. Ringelberg, N.W. MacDonald, K.S. Pregitzer and D.C. White. 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Sci. Soc. Am. J. 61:475–481.