

Microbially available carbon in buried riparian soils in a glaciated landscape

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Received 20 February 2007; received in revised form 27 June 2007; accepted 8 July 2007

Available online 9 August 2007

Abstract

Buried horizons and lenses in riparian soil profiles harbor large amounts of carbon relative to the surrounding soil horizons. Because these buried soil horizons, as well as deep surface horizons, frequently lie beneath the water table, their impact on nitrogen transport across the terrestrial–aquatic interface depends upon their frequency and spatial distribution, and upon the lability of associated organic matter. We collected samples of 51 soil horizons from 14 riparian zones Rhode Island, USA, where soil profiles are characterized by glacial outwash and alluvial deposits. These soil samples came from as deep as 2 m and ranged in carbon content from <1% to 44% in a buried O horizon 54–74 cm deep. We used these samples to: (1) determine the extent to which carbon in buried horizons, and deep surface horizons, is potentially microbially available; (2) identify spatial patterns of carbon mineralization associated with surface and buried horizons; and (3) evaluate likely relationships between soil horizon types, chemical characteristics and carbon mineralization. Carbon mineralization rates associated with buried horizons during anaerobic incubations ranged from 0.0001 to 0.0175 $\mu\text{mol C kg soil}^{-1} \text{ s}^{-1}$ and correlated positively with microbial biomass ($R = 0.89$, $P < 0.0001$, $n = 21$). Excluding surface O horizons from the analysis, carbon mineralization varied systematically with horizon type (surface A, buried A, buried O, lenses, A/C, B, C) ($P < 0.05$) but not with depth or depth \times horizon interaction (overall $R^2 = 0.59$, $P < 0.0005$, $n = 47$). In contrast to this result and to most published data sets, ^{13}C -to- ^{12}C and ^{15}N -to- ^{14}N ratios of organic matter declined with depth (^{13}C –26.9 to –29.3 per mil, ^{15}N +5.6 to –0.8 per mil). The absence of a relationship between horizon depth and C availability suggests that carbon availability in these buried horizons may be determined by the abundance and quality of organic matter at the time of horizon formation or burial, rather than by duration since burial, and implies that subsurface microbial activity is largely disconnected from surface ecosystems. Our results contribute to the emerging view that buried horizons harbor microbially available C in quantities relevant to ecosystem processes, and suggest that buried C-rich soil horizons need to be incorporated into assessments of the depth of the biologically active zone in near-stream subsurface soils.

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Keywords: Alluvium; Groundwater; Biogeochemistry; Rhode Island; Glacial outwash; Denitrification; Eutrophication; Lithology; Riparian zones; Organic matter; Microbial biomass; Stable isotopes; Carbon; ^{13}C ; ^{15}N ; Subsurface

1. Introduction

Recent attempts to develop functional classifications of riparian zones have used hydrogeology as an organizing principle, and have underscored the need to identify landscape settings where groundwater flow paths intersect concentrations of microbially available organic carbon (Jordan et al., 1997; Devito et al., 2000; Puckett, 2004; Vidon and Hill, 2004; Kellogg et al., 2005). At the same

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time, the emerging picture of subsurface soils and sediments along the upland-riparian-stream continuum has pointed towards carbon-rich, buried horizons in riparian zones as “hot spots” of microbial activity (Hill and Cardaci, 2004; Hill et al., 2004). While the presence of these soil horizons has long been noted, their importance to biological activity has received relatively little attention, particularly given their ubiquitous presence in alluvial settings. There is particular interest in the ability of these horizons to support denitrification in the riparian subsurface, where numerous studies have found that the supply of microbially available carbon (C) exerts strong control on microbial activity (Groffman et al., 1992, 1996; Hill, 1996; Lowrance et al., 1997; Martin et al., 1999; Burt et al., 2002; Kellogg et al., 2005).

The impact of buried horizons on subsurface microbial activity, and on associated ecosystem processes such as nitrogen (N) transport, depends upon their frequency, hydraulic conductivity, and spatial distribution, and upon the extent to which associated soil organic matter is physically or chemically protected. Buried channel deposits appear to be ubiquitous subsurface features in riparian zones surrounded by level terrain. Hill and Cardaci (2004) and Hill et al. (2004) reported buried soil horizons in 4 of 5 riparian study sites in Ontario, Canada. Similarly, Well et al. (2005) described fluvisols in Germany containing dark humic colors, woody debris, and buried peat deposits, with some profiles showing increased organic matter content and denitrification capacity between 100 and 300 cm. A survey of 22 riparian zones of first to fourth order streams in Rhode Island revealed >280 A, A/C, or C/A soil horizons within 50 cm of the surface, and 66 of these horizons between 100 and 200 cm of the surface (Blazewski, unpublished observation). Frequently, these horizons occupied positions in the soil profile adjacent to sands and gravels, creating opportunities at multiple depths for groundwater flow paths to intersect biologically active zones with a supply of electron donors.

These observations about buried horizons raise several key questions about the extent and distribution of microbially available C associated with them. First, does microbial activity in buried horizons vary predictably with soil horizon type or soil chemistry? We expect soil horizons with more carbon (A, Ab) to have more microbially available C and higher levels of microbial activity than C/A, A/C, or B horizons.

Second, do shallower (and younger) buried horizons support higher levels of microbial activity than those deeper in the profile? If these localized landscape features act as hot spots of carbon mineralization and denitrification within riparian zones, then the distribution of those hot spots with respect to groundwater flow paths mediates their affect on landscape-scale N removal. In addition, aboveground components of ecosystems may interact more with relatively shallow buried horizons than with those deeper in the soil profile.

We expect organic matter in older, deeper buried horizons to be more decomposed compared to younger, shallower horizons. If this is the case, then deeper horizons should also be more humified, enriched in alkyl groups (Sollins et al., 1996), and have lower C:N ratios than less processed organic matter. Previous studies, focused mostly on shallow soil horizons, have found that ^{13}C and ^{15}N ratios in soil organic matter tend to become enriched with depth (e.g., Nadelhoffer and Fry, 1988; Hogberg, 1997). If this pattern holds along depth gradients of buried horizons, then deeper horizons should have enriched ^{13}C and ^{15}N ratios compared to surface soils. Finally, if deeper horizons are more decomposed compared to shallow horizons, they should have less microbially available C.

Third, how sustainable is carbon mineralization associated with buried soil horizons? Radiocarbon dating showed buried soils collected at 83–93 and >250 cm depth to be thousands of years old (Blazewski et al., 2005), and those deeper in the soil profile are clearly older than those nearer the surface. The persistence of this organic matter over millennia raises questions about controls on its decomposition. If decomposition has been impeded by a paucity of electron acceptors, then sharp increases in electron acceptor abundance from anthropogenic nitrate inputs might lead to relatively rapid depletion of these deep C pools.

The goal of this study was to contribute to a general understanding of riparian C dynamics that may affect denitrification in subsurface soils where anthropogenic activity results in nitrate-enriched groundwater. Our objectives were to: (1) determine the extent to which C in buried horizons in Rhode Island is microbially available; (2) identify spatial patterns of carbon mineralization associated with buried horizons; and (3) evaluate likely relationships between soil horizon types, chemical characteristics and carbon mineralization in buried horizons. We addressed these objectives using laboratory studies of a large number of buried soil horizons from many riparian zones in Rhode Island, USA. Nitrogen loads in groundwater to these sites are generally very low ($< 0.1 \text{ mg l}^{-1}$) (Kellogg et al., 2005); our experiments were designed to assess how microbial activity associated with different soils might respond should nitrate loads increase, as they have at many sites in similar hydrogeologic settings.

2. Materials and methods

2.1. Site descriptions

We collected soil samples from riparian forests along 14 stream reaches in the Pawcatuck Watershed, Rhode Island, USA. Surficial geology reflects the region's recent glacial history; all sites in this study were characterized by glacial outwash, often covered by alluvial deposits. Outwash covers approximately 20% of the Rhode Island landscape (Rector, 1981), and these settings should be similar to other outwash settings in the glaciated northeast

(Larson and Stone, 1982). *Acer rubrum* dominated the overstory vegetation at all sites; common understory species included sweet pepperbush (*Clethra alnifolia*) and highbush blueberry (*Vaccinium corymbosum*). Soil drainage classes ranged from very poorly drained (VPD) to somewhat poorly drained (SPD), and sampling occurred between 0.5 and 31 m from the stream edge (Table 1). At four sites for which we had temporal records of water table depth, the summer minimum ranged from 46 to 98 cm below the surface, and the dormant season maximum ranged from 12 cm below the surface to 13 cm above the surface, with a greater range at sites in alluvial compared to outwash settings (Kellogg et al., 2005). A previous study at one site used more intensive hydrologic measurements and also documented water table fluctuations between the dormant and growing seasons (Nelson et al., 1995).

2.2. Sample collection

Soil samples were collected using a standard bucket auger, separated into different horizons in the field, and transported to the laboratory in a cooler. Typically, we collected 0.5-to-1 kg of material per horizon from a single auger hole at each location.

Soil sample collection occurred during three field campaigns in summer 2001, fall 2001, and September 2003, respectively. We collected samples of 51 soil horizons from 14 sites (Table 2), and stored samples at 4 °C until they were either dried or incubated. We mixed samples thoroughly immediately prior to subsampling for laboratory analyses; in accord with the generally sandy texture, none of our samples had aggregates. All the soils we sampled sit in the permanently saturated zone except for the shallower ones that are saturated during the dormant season but not during the growing season. The deepest horizons used in this study came from 210 cm beneath the soil surface.

We designated soils as: O (surface), Ob (buried), A, Ab (buried), A/C, C/A, B, or C horizons, or lenses. We defined lenses as bands of soil <2 cm thick that were enriched in organic matter compared to the surrounding matrix (Fig. 1a). Soil horizons with numerous lenses were often designated as A/C or C/A. To receive the designation of buried (Ob or Ab), the horizon needed to occur beneath a B or C horizon (Fig. 1b). In general, buried horizons occurred at greater depths than surface O and A horizons, but in some cases surface O and A horizons had a combined depth of 1 m.

2.3. Laboratory incubations: carbon mineralization

We incubated between 2 and 40 g of soil in vitro, anaerobically, varying the amount of soil inversely with visual estimates of C abundance and previously identified horizon type (e.g., O, Ab, C). Because our objective was to assess carbon availability under conditions of enhanced N loads, we also added 20 mg $\text{NO}_3\text{-N kg soil}^{-1}$ to each jar, in

10 ml of deionized water. We altered this procedure for some of the incubations we used to assess the influence of nitrate and DOC amendments on C mineralization, as explained below. To establish anaerobic conditions, jars were sequentially evacuated and flushed with N_2 gas at least three times and then brought to atmospheric pressure using a water trap. We kept jars in the dark at ~20 °C and measured the CO_2 concentration in the headspace of each jar after 1, 7, 14, and 28 days on a Varian 3400X gas chromatograph equipped with a thermal conductivity detector.

To assess the influence of nitrate availability and DOC from forest floor leachate on microbial activity in the subsurface, we incubated three replicate jars of six soil samples. We selected these samples to span a range of sites (5), C content (A/C, Ab, and O) and depths (0–115 cm). For each soil, one jar received DI with nitrate, one received only deionized water (DI), and one received filtrate from forest floor shaken in DI for 1 h and passed through a GF/F filter.

After concluding the incubation studies, we oven-dried the entire sample in each jar at 60 °C for a minimum of three days and weighed it to obtain a sample-specific measurement of total soil mass.

2.4. Soil chemistry

We measured soil carbon and nitrogen content, ^{13}C , and ^{15}N on a subset of 18 samples, including samples of all horizon types except for surface O horizons. We removed most roots, macroscopic organic matter fragments, and rocks, and then ground samples to a fine powder using a freezer mill. Samples were analyzed at the Cornell Stable Isotope Laboratory on a Finnigan MAT Delta Plus stable isotope ratio mass spectrometer running in continuous flow mode.

2.5. Microbial biomass

We used chloroform fumigation extraction (Paul et al., 1999) to measure microbial biomass of the samples collected during September 2003. We fumigated one subsample of each soil sample in a sealed dessicator for 24 h. We then extracted it and a paired subsample with 0.5 M K_2SO_4 and filtered resulting extracts through preashed GF/F filters. Filtrate was analyzed for DOC on a Shimadzu TOC-5050 analyzer at McGill University using high temperature combustion with a platinum catalyst at 680 °C and an IRGA, with up to six analytical replicates per sample. We calculated microbial biomass C using a value of 0.35 for K_{ec} (Horwath and Paul, 1994).

2.6. Data analysis

For data analysis, we used JMP IN 5.1 and SAS 9.1.3 (SAS Institute, Cary, NC). We tested for differences among sample groups using 1-way ANOVAs. We used regression

Table 1
 Characteristics of streams and sites where we sampled buried horizons, nutrient chemistry of soil samples, and carbon mineralization rates of each incubated soil sample.

Site ^a	Exp ^b	Strm Ord. ^c	Dist. Strm (m) ^d	Soil Drain ^e	Pb (g cm ⁻³)	Pent C	Pent N	C-min per soil ^f	C-min per C ^g	C-N Ratio	delta ¹³ C ^h	delta ¹⁵ N ⁱ	Depth (cm)	Hor. Type ^j	Width (cm)
BR 1	1	3	20	VPD	ND	ND	ND	0.1128	ND	ND	ND	ND	6.5	O	13
BR 1	1	3	20	VPD	ND	ND	ND	0.0063	ND	ND	ND	ND	74	A	12
BR 3	1	3	10	PD	ND	ND	ND	0.0175	ND	ND	ND	ND	54	Ab	18
BR 3	3	3	0.5	VPD	ND	ND	ND	0.0127	ND	ND	ND	ND	21	A	42
BR 3	3	3	0.5	VPD	ND	ND	ND	0.0084	ND	ND	ND	ND	51	A	18
BR 3	3	3	0.5	VPD	ND	ND	ND	0.0123	ND	ND	ND	ND	69.5	A	19
BR 3	3	3	0.5	VPD	ND	ND	ND	0.0110	ND	ND	ND	ND	104.5	A	51
BR 4	1	3	5	VPD	ND	ND	ND	0.0008	ND	ND	ND	ND	77.5	C/A	35
BR 4	1	3	5	VPD	ND	ND	ND	0.0002	ND	ND	ND	ND	106	C	22
BR 4	1	3	5	VPD	ND	ND	ND	0.0002	ND	ND	ND	ND	157.5	C	35
BR 5	1	3	20	VPD	ND	ND	ND	0.0274	ND	ND	ND	ND	13.5	A	7
BR 5	1	3	20	VPD	ND	ND	ND	0.0219	ND	ND	ND	ND	30	A	26
BR 5	1	3	20	VPD	ND	ND	ND	0.0299	ND	ND	ND	ND	61	A	36
BR 5	2	3	1	VPD	ND	0.5	0.02	0.0017	0.3400	20	-28.6	0.62	97.5	A/C	25
BG	2	1	ND	PD	ND	8.3	0.37	0.0022	0.0267	22	-27.9	-0.01	197.5	Ab	25
CH	1	1	3	PD	ND	ND	ND	0.0018	ND	ND	ND	ND	30	B	26
CH	1	1	3	PD	ND	ND	ND	0.0005	ND	ND	ND	ND	62	C	14
CH	1	1	3	PD	ND	ND	ND	0.0011	ND	ND	ND	ND	74.5	A/C	11
LL	2	1	7	VPD	0.71	6.0	0.14	0.0002	0.0034	43	-27.7	3.91	46	A	2
LL	2	1	7	VPD	0.80	4.9	0.20	0.0003	0.0059	26	-28.1	2.81	69	A	10
LL	1	1	2	VPD	ND	8.5	0.21	0.0006	0.0071	41	-28.2	4.23	71.5	Lenses	9
MB	3	2	10	PD	ND	ND	ND	0.0892	ND	ND	ND	ND	2.5	O	5
MB	3	2	10	PD	0.32	ND	ND	0.0693	ND	ND	ND	ND	7	O	4
MB	3	2	10	PD	0.74	6.1	0.52	0.0215	0.3530	12	-26.9	5.56	14.5	A	11
MB	3	2	10	PD	1.29	ND	ND	0.0024	ND	ND	ND	ND	31	B	22
MB	3	2	10	PD	1.11	ND	ND	0.0024	ND	ND	ND	ND	45.5	Ab	7
MB	3	2	10	PD	1.50	ND	ND	0.0017	ND	ND	ND	ND	55.5	A/C	13
MB	3	2	10	PD	1.40	ND	ND	0.0015	ND	ND	ND	ND	74	A/C	24
MB	3	2	10	PD	1.16	6.6	0.27	0.0041	0.0617	25	-28.9	-1.07	94.5	Ab	17
MB	3	2	10	PD	1.36	ND	ND	0.0013	ND	ND	ND	ND	109	A/C	12

MB	2	2	10	PD	ND	3.0	0.13	0.0018	0.0592	23	−29.0	0.38	125	Lenses	10
MB	3	2	10	PD	1.31	ND	ND	0.0013	ND	ND	ND	ND	127.5	A/C	25
MB	3	2	10	PD	1.71	ND	ND	0.0017	ND	ND	ND	ND	155	C	30
PB	1	3	31	PD	1.30	ND	ND	0.0015	ND	ND	ND	ND	35	Ab	8
PB	2	3	31	PD	1.03	2.8	0.12	0.0004	0.0142	24	ND	ND	53.5	Ab	7
PB	2	3	31	PD	0.47	7.0	0.35	0.0015	0.0214	20	−29.1	0.60	88	O	10
PB	2	3	31	PD	0.72	9.1	0.38	0.0024	0.0263	24	−28.2	1.20	110	O	8
WHB	2	2	0.5	VPD	ND	43.9	1.49	0.0051	0.0116	30	−27.7	1.95	64	O	20
WHB	2	2	0.5	VPD	ND	8.7	0.32	0.0017	0.0195	27	−28.4	−0.08	93.5	Ab	5
WHB	2	2	0.5	VPD	ND	31.7	1.10	0.0060	0.0189	29	−27.9	−0.43	93.5	O	5
WR 1	1	4	1	PD	ND	ND	ND	0.0028	ND	ND	ND	ND	80	Lenses	ND
WR 2	3	4	10	PD	ND	ND	ND	0.2168	ND	ND	ND	ND	2	O	4
WR 2	3	4	10	PD	ND	ND	ND	0.0058	ND	ND	ND	ND	11.5	A	15
WR 2	3	4	10	PD	ND	ND	ND	0.0014	ND	ND	ND	ND	24.5	C/A	11
WR 2	3	4	10	PD	ND	ND	ND	0.0060	ND	ND	ND	ND	56	Ab	16
WR 2	3	4	10	PD	ND	12.6	0.73	0.0119	0.0950	17	−27.9	0.74	100.5	Ab	29
WR 2	3	4	10	PD	ND	1.5	0.06	0.0032	0.2206	23	−29.3	−0.76	123.5	A/C	17
WR 4	2	4	0.5	SPD	ND	ND	ND	0.0005	ND	ND	ND	ND	48.5	Ab	7
WR 4	2	4	0.5	SPD	ND	9.4	0.3	0.0005	0.0053	31	−29.3	ND	202.5	Ab	11
YR	2	1	5	VPD	ND	1.5	0.05	0.0001	0.0066	29	−27.5	ND	58.5	Lenses	17
YR	1	1	5	VPD	ND	ND	ND	0.0036	ND	ND	ND	ND	107.5	Ab	15

Sites are described in more detail in Blazewski et al. (2005) and Kellogg et al. (2005).

^aSites with the same name followed by different numbers indicate different reaches of the same stream.

^bExperiment (or incubation batch).

^cStream order.

^dDistance from stream to auger hole.

^eSoil drainage class. PD, poorly drained; SPD, somewhat poorly drained; VPD, very poorly drained.

^fC mineralization per mass of soil ($\mu\text{mol CO}_2 \text{ kg soil}^{-1} \text{ s}^{-1}$).

^gC mineralization per gram of soil carbon ($\mu\text{mol CO}_2 \text{ kg C}^{-1} \text{ s}^{-1}$).

^hPer mil, relative to Pee Dee Belemnite.

ⁱPer mil relative to atmospheric air.

^jHorizon type.

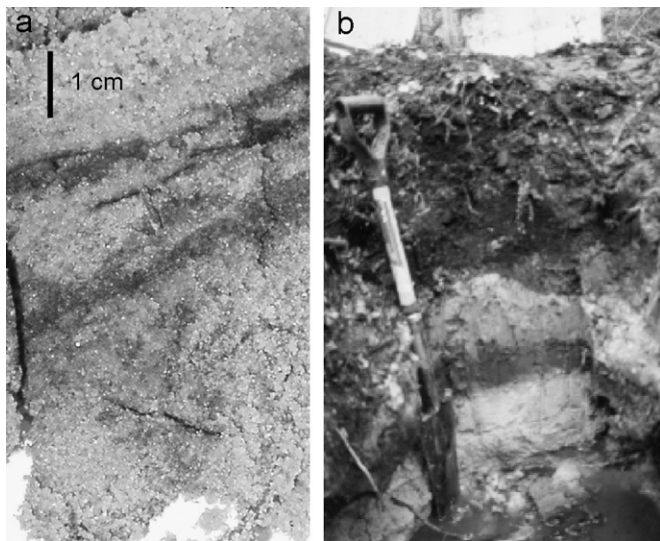


Fig. 1. (a) An A/C horizon illustrating lenses and (b) a buried horizon.

Table 2
Frequency of soil horizons sampled by depth and type.

Depth (cm)	O	Ob	A	Ab	B & C	Lenses	A/C & C/A	Total
<25	4		4				1	9
25–49			2	3	2			7
50–99		3	5	5	1	3	5	22
100–150		1	1	2	1	1	3	9
>150				2	2			4
Total	4	4	11	12	6	4	9	51

and correlation analyses to explain relationships between soil chemistry and microbial activity. In building regressions, we first identified terms that clearly did and did not contribute explanatory power and used this understanding to construct the most informative set of models.

3. Results

3.1. Carbon mineralization

Carbon dioxide accumulated steadily over the 28-day incubations (Fig. 2). While Fig. 2 shows only data from samples collected in September 2003, we observed a similar pattern in incubations from other sample dates. For most soil samples, carbon mineralization rates were similar at the beginning and end of the incubation, diminishing only slightly. Carbon dioxide production in soils with high carbon mineralization rates (surface A and O horizons) declined between 200 and 400 h and then increased, while carbon mineralization rates associated with Ab and low-carbon soils exhibited a slow decline over the course of the study (Fig. 2).

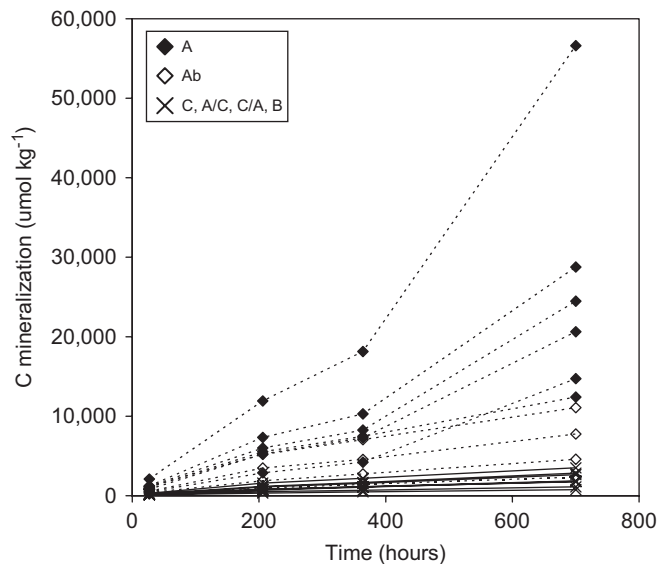


Fig. 2. CO₂ accumulation during incubations of 21 riparian soil horizons (A, Ab, B, C, A/C, C/A) collected in September 2003. Samples from O horizons (not shown) mineralized 150,000–360,000 μm CO₂ kg⁻¹ by hour 700.

3.2. Soil depth and horizon type

Apart from surface O horizons, which had carbon mineralization rates much higher than other soils, we found no relationship (ANOVA, $P > 0.18$, $n = 18$) between carbon mineralization rate and soil depth in the samples taken in September 2003. However, we did observe differences in carbon mineralization rates among different soil horizons (ANOVA, $P < 0.0005$, $n = 21$). Carbon mineralization rates were highest in surface O horizons and declined in the sequence: A > Ab > C, A/C, C/A, and B horizon types (Fig. 2). Similarly, an ANOVA model applied to data excluding surface O horizons showed that horizon type ($P < 0.01$) explained a significant proportion of variance in carbon mineralization, but depth ($P > 0.4$) and horizon type × depth interaction ($P > 0.4$) did not (overall model $R^2 = 0.67$, $P < 0.05$, $n = 18$).

To further explore relationships between soil characteristics and carbon mineralization in the riparian subsurface, we combined data from samples taken in September 2003 with data from samples collected in 2001 (Fig. 3). An ANOVA run on the entire data set with carbon mineralization as the dependent variable and depth, horizon type, horizon type × depth interaction, and sample batch (i.e., collection date) as independent variables yielded an R^2 of 0.77 (overall model $P < 0.0001$, $n = 51$). This analysis showed strong effects of soil horizon type ($P < 0.05$), depth ($P < 0.05$), and horizon × depth interaction ($P < 0.0001$), but not sample batch ($P > 0.5$).

When surface O horizons were excluded from the analysis, horizon type ($P < 0.05$) remained a significant predictor of C mineralization, but soil depth ($P > 0.5$) and depth × horizon ($P > 0.5$) did not (overall $R^2 = 0.59$,

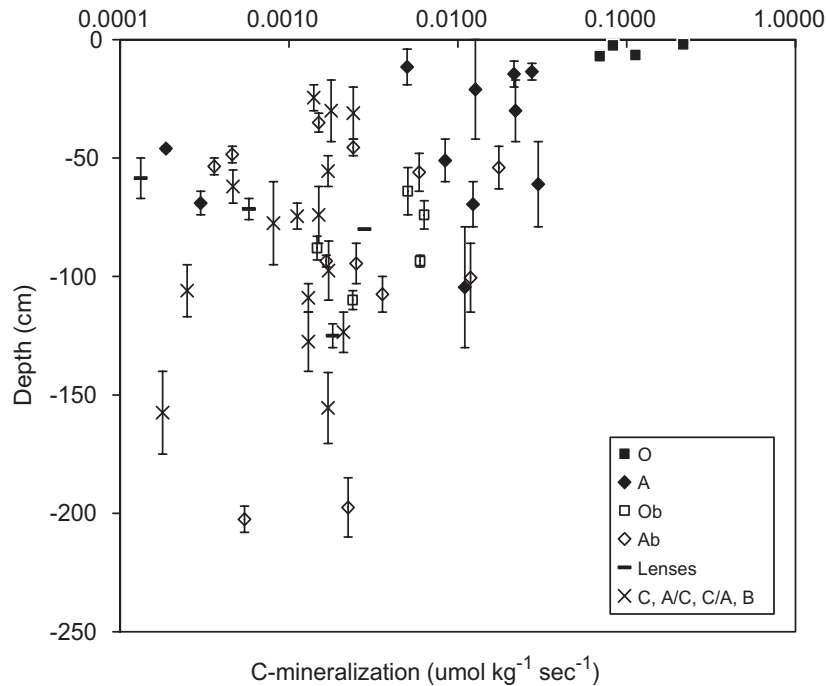


Fig. 3. Carbon mineralization versus depth of riparian forest soil horizons during anaerobic incubations amended with KNO_3 . Vertical bars indicate the thickness of each soil horizon, not measurement error. Note log scale on x -axis.

$P < 0.0005$, $n = 47$), indicating that the predictive value of depth in the earlier analysis derived from the high C mineralization rates associated with surface O horizons and likely does not apply in the subsurface. Sample batch also emerged as a significant predictor ($P < 0.05$). The influence of sample batch derived from the relatively high C mineralization rates of samples collected in fall 2003 and may reflect the shorter storage times of those samples compared to others used in this study.

3.3. Microbial biomass

Microbial biomass measured on fresh samples mirrored the relationship of carbon mineralization to soil horizon type. We found greatest biomass in O horizons, intermediate levels in A and Ab horizons, and lowest levels in C and C/A horizons (Fig. 4). A model including depth, horizon type, and depth \times horizon type interaction, applied to the data excluding surface O horizons, showed a significant effect of horizon type ($P < 0.05$) but not of depth ($P > 0.5$) or horizon type \times depth interaction ($P > 0.15$). We found a strong correlation between microbial biomass and carbon mineralization rate (Fig. 5, $R = 0.89$, $P < 0.0001$, $n = 21$) for nontransformed data.

3.4. Response to amendments

Carbon mineralization declined in response to nitrate amendments ($P = 0.05$, 2-tailed paired T -test), but these differences were small compared with variation in carbon mineralization owing to innate characteristics of six soil

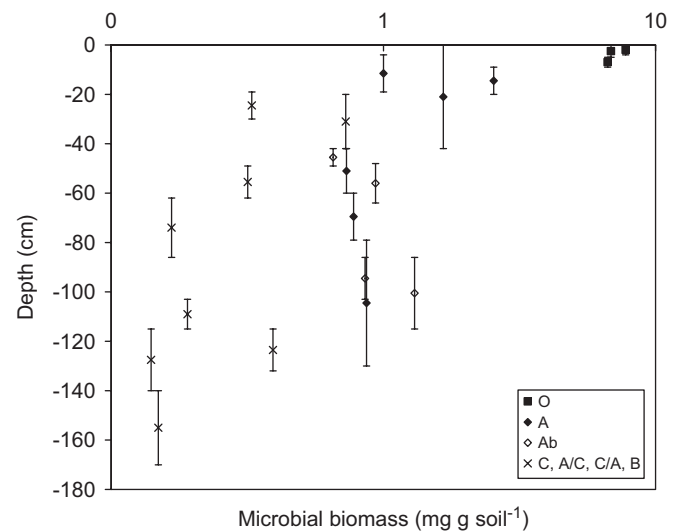


Fig. 4. Microbial biomass versus depth in 21 riparian soil horizons (O, A, Ab, B, C, A/C, C/A) collected in September 2003. Note log scale on x -axis.

horizons varying in depth and site of origin. Amending soil incubations with DOC did not influence carbon mineralization ($P > 0.3$, 2-tailed paired T -test).

3.5. Soil C and N

Soil carbon content ranged from $< 1\%$ in some C horizons to 44% in a buried O horizon 54–74 cm below the soil surface (Table 1). Soil nitrogen ranged from 0.1% in

some lenses and A/C horizons to 1.25% in the 54–74 cm deep buried O horizon (Table 1).

Together, soil carbon and nitrogen content explained 78% of the variation in carbon mineralization rates in these incubations ($P < 0.0001$, $n = 18$). In this model, both C content and N content appeared as significant predictors ($P < 0.005$) but C-to-N ratio did not ($P > 0.5$). Correlations with C mineralization rate were not significant for C content, marginally significant for N content, and clearly significant for C-to-N ratio (Table 3). However, the latter two relationships derived largely from a small number of points; in particular, when we excluded two points with low C-to-N ratios from the analysis, the correlation between C mineralization rate and C-to-N ratio disappeared ($P > 0.2$). A parallel analysis using C mineralization per mass of soil C yielded similar but weaker patterns.

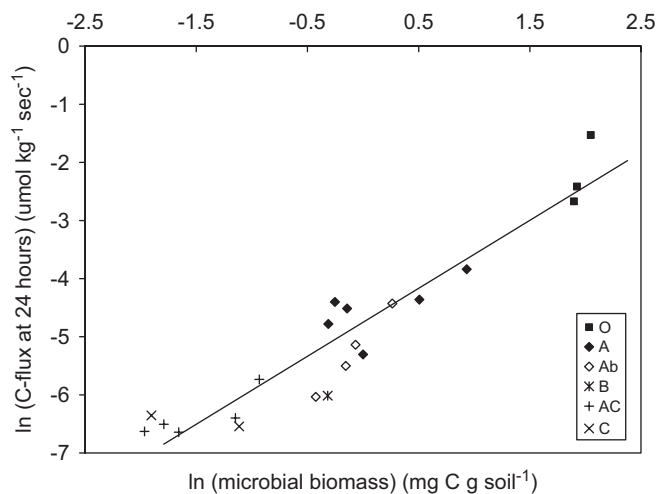


Fig. 5. Carbon mineralization versus microbial biomass in 21 riparian soil horizons (O, Ab, B, C, A/C, C/A) collected in September 2003.

Table 3

Pearson correlation coefficients showing relationships among variables characterizing soil microbial activity, chemistry, and location. Sample size shown in parentheses.

Variable	C-min per soil ^a	C-min per C ^b	Pent C	Pent N	C-to-N	delta ¹⁵ N	delta ¹³ C
C-min per soil ^a	1						
C-min per C ^b	0.72 (19)**	1					
Pent C	0.19 (18) ⁺	-0.17 (18) ⁺	1				
Pent N	0.42 (18) ^S	0.03 (18) ⁺⁺	0.96 (18)***	1			
C-to-N	-0.60 (18)*	-0.65 (18)**	0.17 (18) ⁺	-0.05 (18) ⁺⁺	1		
delta ¹⁵ N	0.35 (15) ⁺	0.16 (15) ⁺⁺	-0.03 (15) ⁺⁺	-0.002 (15) ⁺⁺	0.23 (15) ⁺	1	
delta ¹³ C	0.52 (17)*	0.24 (17) ⁺	0.29 (17) ⁺	0.38 (17) ^{SS}	-0.01 (17) ⁺⁺	0.69 (15)*	1
Depth	-0.44 (51)**	-0.16 (19) ⁺⁺	-0.06 (18) ⁺⁺	-0.09 (18) ⁺⁺	-0.03 (18) ⁺⁺	-0.71 (15)**	-0.59 (17)*

^aC mineralization rate per mass of soil ($\mu\text{mol kg soil}^{-1} \text{sec}^{-1}$).

^bC mineralization rate per mass of carbon ($\mu\text{mol kg C}^{-1} \text{sec}^{-1}$).

*** $P < 0.0005$.

** $P < 0.005$.

* $P < 0.05$.

^S $P < 0.1$.

^{SS} $P < 0.2$.

⁺ $P > 0.2$.

⁺⁺ $P > 0.5$.

3.6. Soil ¹⁵N and ¹³C

Soil ¹⁵N and ¹³C generally declined with depth (Fig. 6, Table 3). The correlation between ¹⁵N and depth ($R = 0.71$, $P < 0.005$, $n = 15$) improved ($R = 0.84$, $P < 0.0001$, $n = 14$) when we excluded the single sample deeper than 150 cm. The small number of horizons from beneath 150 cm included in this data set precludes the ability to extend the analysis of either element to greater depths. Soil ¹³C correlated moderately with C mineralization rate ($R = 0.52$, Table 3); this relationship depended on two samples with C mineralization rates $> 0.01 \mu\text{mol C kg}^{-1} \text{sec}^{-1}$.

4. Discussion

4.1. Is microbially available C a general feature of buried horizons in riparian zones?

We found substantial amounts of microbially available C in buried horizons in the riparian subsurface, indicated both by anaerobic laboratory incubations (Fig. 3) and by measurements of microbial biomass on fresh soil (Fig. 4). Some caution is required when drawing inferences about processes under field conditions from laboratory data. Measurements of microbial biomass can include inactive as well as active microbes, and microbes in laboratory incubations may respond to conditions not normally encountered in the field. However, because the soils we studied have minimal structure and no aggregates, and because we did not grind samples, our sampling procedure did not change soil texture, disturb soil structure, or expose surfaces that had been physically protected under field conditions. Similarly, we conducted our incubations under anaerobic conditions, like those that occur in the field. We intentionally elevated nitrate concentrations in

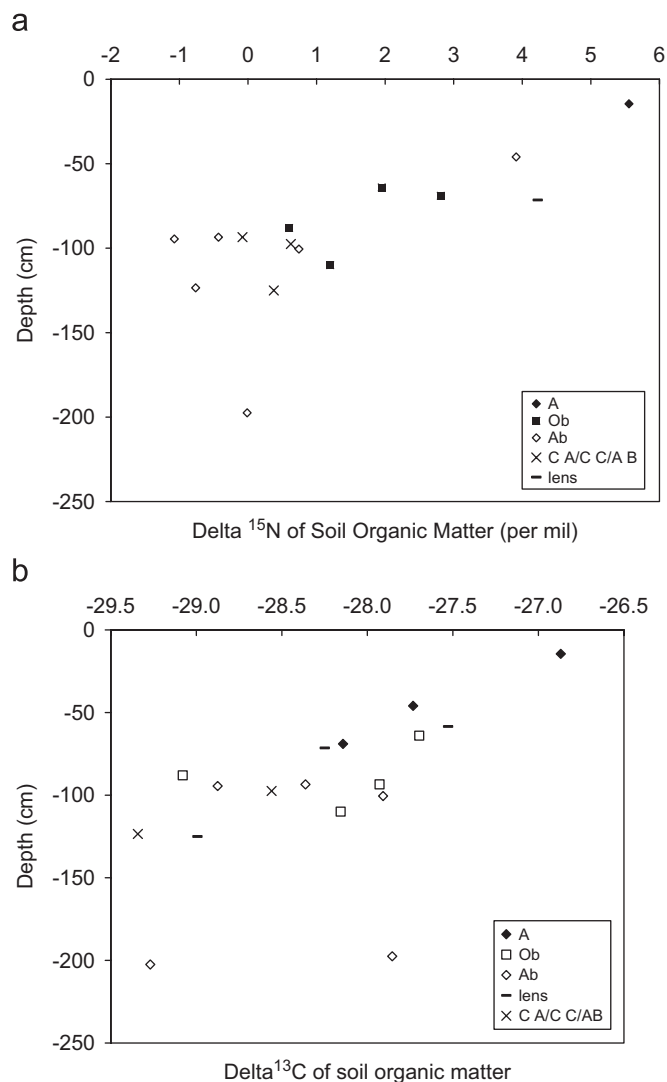


Fig. 6. Spatial patterns of (a) delta ^{15}N and (b) delta ^{13}C of soil organic matter from samples collected in summer and fall 2001. Vertical bars indicate the thickness of each soil horizon.

our laboratory incubations because we sought insight about what would happen to carbon associated with buried horizons if nitrate flows were to increase. The consistent response in C mineralization and microbial biomass (Fig. 5, Table 3) suggests that our assays reflect the distribution of microbially available C and associated microbial activity in buried horizons in shallow groundwater beneath riparian zones in Rhode Island.

Our results, based on incubations of 51 soil horizons from 14 sites, support the emerging view that microbially available C is a general feature of buried horizons in riparian zones. In terms of carbon content, the buried horizons we studied in Rhode Island (<1–44% C) span a somewhat wider range than buried horizons studied in Ontario (5–21% organic matter) (Hill et al., 2004). The buried horizons we studied are also thousands of years old (Blazewski et al., 2005), whereas those described by Hill et al. (2004) appear to post-date European settlement. The

carbon mineralization rates we measured closely resemble those reported by Hill and Cardaci (2004).

4.2. Controls on carbon mineralization in buried horizons

Carbon availability in these buried horizons may be determined ultimately by abundance and quality of organic matter at the time of horizon formation or burial, rather than by duration since burial or the influence of contemporary surface ecosystems. Variation in quality and quantity of organic matter at the time of burial could have resulted from shifts in plant community composition at these sites over time, with some plants having more recalcitrant litter than others. Alternatively, current stocks of microbially available C in buried horizons may reflect physical conditions (e.g., flood strength, flood timing, topography) that controlled organic matter accumulation at the time of burial. For example, in some cases organic matter may have been buried while relatively fresh (forming buried horizons with higher C content and more microbially available C), while in other cases it may have begun to decompose before transport and burial (leading to buried horizons with lower C content and less microbially available C).

Although our data do not allow us to determine conclusively the ultimate controls on C mineralization in the riparian subsurface, they are consistent with the propositions that: (1) current soil organic matter quality depends on the initial organic matter source or conditions during horizon formation; and (2) subsurface microbial activity is largely disconnected from surface ecosystem processes. We observed that carbon mineralization and microbial biomass varied systematically with horizon type but not depth, when we excluded surface O horizons from the analysis. If surface processes were influencing subsurface carbon availability (e.g., via roots or DOC leaching), we would expect subsurface C availability to diminish with depth. Similarly, if organic matter decomposition were resulting in progressively more recalcitrant material, we would expect older (deeper) horizons to have lower C mineralization rates than younger (shallower) horizons. Therefore, our data suggest that the initial character of buried horizons is more important than time since burial or contemporary surface ecosystem function in driving microbial activity in the subsurface of riparian zones in alluvial landscapes.

4.3. Spatial patterns of ^{13}C and ^{15}N in buried horizons

The fact that ^{13}C and ^{15}N ratios tended to decrease with depth in the soil profile (Fig. 6, Table 3) is somewhat surprising because ^{13}C and ^{15}N ratios in soil organic matter typically become enriched with depth (e.g., Nadelhoffer and Fry, 1988; Hogberg, 1997). However, most stable isotope profiles have been restricted to the top 30 cm, and data for riparian soils, saturated soils, and deep soil profiles are rare (Krull et al., 2006; Wynn et al., 2006;

Zhu and Liu, 2006). The contrast underscores the need for caution when interpreting patterns of stable isotope ratios in soil organic matter. Potential mechanisms contributing to the patterns we observed include: organic matter processing over long time scales; water table fluctuations (cyclic hydromorphism); stabilization by association with minerals; compound-specific decomposition; differential loss of isotopes by leaching and dispersion; or differences in stable isotope signatures among soil horizons at the time of burial.

4.4. Persistence of buried horizons

If buried horizons persist in riparian soil profiles due to a paucity of electron acceptors in these saturated, generally anaerobic ecosystems, then an increase in electron acceptor abundance, e.g., NO_3^- , might accelerate decomposition and limit their longevity. Two observations suggest this is not the case. First, dissolved oxygen concentrations measured in groundwater in riparian zones at our sites are often $>2 \text{ mg l}^{-1}$, and even when they are $<1 \text{ mg l}^{-1}$ they are not zero (Simmons et al., 1992; Nelson et al., 1995; Addy et al., 1999; Kellogg et al., 2005). Second, carbon mineralization declined in response to nitrate amendments under anaerobic conditions, consistent with the explanation that N limited microbial growth but not respiration (Bengtson and Bengtsson, 2005). These observations suggest that decomposition rates of buried horizons are unlikely to increase dramatically as N loads in groundwater increase.

Combining our measured rates with estimates of C stocks in buried horizons suggests that rates of C mineralization measured in our laboratory incubations are considerably faster than in situ rates, particularly at the high end of our measurements. Carbon mineralization rates for most of the buried horizons and lenses in this study ranged between 0.0005 and $0.018 \mu\text{mol C kg}^{-1} \text{ s}^{-1}$ (Fig. 3). The carbon content of these horizons and lenses usually fell between 1% and 13%, with bulk densities from 0.7 to 1.4 g cm^{-3} (Table 1). Our measured C mineralization rates, which spanned a wide range (Fig. 3), would exhaust this C stock in between 4 and 690 years.

To obtain an independent estimate of subsurface C mineralization, potentially associated with buried horizons, we calculated the amount of C mineralization required to support denitrification rates measured in situ by Kellogg et al. (2005) at four of our study sites. Kellogg et al. (2005) reported mean denitrification rates between 7 and $140 \mu\text{g N kg}^{-1} \text{ day}^{-1}$ at 65 and 150 cm beneath the surface where groundwater nitrate concentrations had been spiked to $32 \text{ mg NO}_3\text{-N l}^{-1}$. Under these conditions, nitrate reduction likely dominates microbial respiration, and these denitrification rates would require C mineralization rates between 2.9×10^{-5} and $5.8 \times 10^{-4} \mu\text{mol C kg}^{-1} \text{ s}^{-1}$. Assuming buried horizons were the only source of C consumed during Kellogg et al.'s (2005) experiments, and assuming an initial C content of 3–12%, they could persist for 70–6000 years. If other C sources contributed to C

mineralization during these experiments, then the potential longevity of the buried horizons increases accordingly.

4.5. The biologically active zone

While rates of carbon mineralization in surface O horizons were an order of magnitude higher than in other soils we studied, these data and similar studies (e.g., Hill et al., 2004; Well et al., 2005) call into question the view that the biologically active zone is restricted to surface soils. At least in alluvial landscapes such as the ones we studied, buried A horizons, buried O horizons, and deep A horizons occur frequently, particularly at the riparian-stream interface. These soils often harbor labile C and therefore extend the depth of the biologically active zone well below the surface. This assessment of the biologically active zone in the riparian subsurface parallels two recent examinations of deep soil in other contexts. Reconsideration of data on biotic activity in deep upland profiles led soil scientists to include the C horizon in the concept of soil (Richter and Markewitz, 1995), and data on root distributions have led ecosystem scientists to extend the concept of rooting depth to tens of meters in seasonally dry forests (e.g., Nepstad et al., 1994; Trumbore et al., 1995). In all three cases, low rates of biological activity on a per-volume basis have important consequences for ecosystem fluxes when summed over relevant parts of the soil profile, and in the case of the riparian subsurface, low rates of C mineralization can support ecosystem-relevant rates of N removal.

Considerable concern has emerged about the extent to which water crossing the terrestrial–aquatic interface may bypass the biologically active region of riparian zones, either by flowing over the surface (Warwick and Hill, 1988) or along deep flow paths (Bohlke and Denver, 1995; Vidon and Hill, 2004). Such disconnections do appear to occur, as illustrated by sites in Oregon, USA, where most of the water in the creek reaches the channel via overland flow (Wigington et al., 2003). Gold et al. (2001) invoked a similar model to suggest that fine-grained sediments in glacial till create seeps, again leading to overland flow and eliminating the potential for subsurface denitrification.

Our data about microbially available C in the subsurface suggest that deep flow paths may not always negate the potential for riparian processing of upland-derived NO_3^- , and in situ measurements of denitrification at four intensively studied sites (Kellogg et al., 2005) are consistent with this view. Carbon-rich horizons accounted for over 50% of the cross-sectional area of the soil profile at some of our study sites (Blazewski, unpublished observation), underscoring the potential for flow paths to intersect C-rich soil horizons. Further, Jacinthe et al. (1998) found that groundwater flows into organic matter patches in the subsurface, and demonstrated that these patches accumulate N carried in groundwater. Given that microbially available C associated with buried horizons and lenses did not diminish systematically with depth (Figs. 3, 4), that we

found buried horizons in 23 of 24 riparian zones surveyed, that 30% of these horizons occurred deeper than 50 cm, and that 18% occurred deeper than 1 m, there appears to be an abundance of opportunities for N-rich groundwater moving with the top 2 m of the soil profile to intersect biologically active zones in these landscapes.

5. Conclusions

Carbon mineralization rates from incubations of soil samples collected from 51 soil horizons at 14 sites, and measurements of microbial biomass on a subset of those samples, support the emerging view that microbially available C is a general feature of buried horizons in riparian zones. Buried horizons that vary substantially in location, organic matter content, and age harbor quantities of microbially available C relevant to subsurface ecosystems.

Soil horizon type (Oa, Ob, A, Ab, A/C, B, C, lenses) and soil C and N chemistry—but neither soil depth nor depth \times horizon type interaction—explained a substantial proportion of variation in C mineralization rate among samples. Although our data do not allow us to determine conclusively the ultimate controls on C mineralization in the riparian subsurface, these patterns are consistent with the propositions that: (1) current soil organic matter quality depends on the initial organic matter source or conditions during horizon formation; and (2) subsurface microbial activity is largely disconnected from surface ecosystem processes.

The pattern of declining ^{13}C and ^{15}N with depth that we observed contrasts most published soil profiles of these stable isotope signatures and raises questions about the underlying cause. Potential contributing mechanisms include significant amounts of organic matter processing by low rates of activity operating over millennia, water table fluctuations (cyclic hydromorphism), compound-specific decomposition and differential loss of isotopes by leaching and dispersion.

Our data showing the widespread presence of microbially available C in buried horizons suggest that the biologically active zone extends well below the surface in alluvial riparian zones in glaciated landscapes, and imply that the potential for deep flow paths to bypass riparian processing of upland-derived nitrate may be relatively low in this hydrogeologic setting.

Acknowledgments

We thank Esther Pullen, Ashley Wilson, Krista Guerrero, and Melanie Hayn for assistance in the laboratory. Mike Dalva and Tim Moore at McGill University graciously analyzed the soil extracts used to estimate microbial biomass. Barbara Bedford and Tim Fahey provided insights during many phases of this research, and helpful comments on an earlier draft of this manuscript. This research was supported by grants from the

USDA, an NSF IGERT to the Cornell Program in Biogeochemistry and Environmental Biocomplexity, and the Andrew W. Mellon Foundation.

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