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August 7, 2009

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**AN EVALUATION OF BACTERIAL AND FUNGAL CONTRIBUTIONS TO
ORGANIC MATTER DECOMPOSITION ALONG A SOIL MOISTURE
GRADIENT**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science in Biology at Virginia Commonwealth University.

by

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Abstract

AN EVALUATION OF BACTERIAL AND FUNGAL CONTRIBUTIONS TO ORGANIC MATTER DECOMPOSITION ALONG A SOIL MOISTURE GRADIENT

By Morgan Alice Rawls, MS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

Virginia Commonwealth University, 2009

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The decomposition of plant litter is a critical biological function that aids in nutrient cycling and energy transfers within and between ecosystems. The primary decomposers of dead leaf material are bacteria and fungi, though there is no consensus as to which of these groups is dominant, nor is it known how the abundance and composition of these communities changes over time. The objectives of this study were to examine the relative contributions of bacterial and fungal populations to leaf organic matter (OM) decomposition and to consider the effect of moisture availability on the microbial community. The study was conducted across three habitats of differing moisture regimes:

an upland terrestrial site, an emerging freshwater marsh, and an established freshwater swamp. Litterbags were constructed using two types of vegetation: a standardized substrate, maple leaves, and the site-specific vegetation, deployed in November 2007 following plant senescence, and retrieved after 0, 3, 6, 10, and 16 months of field incubation. The samples were then analyzed for decomposition as % OM remaining, total carbon and nitrogen content (C:N), dissolved organic carbon (DOC) release, microbial respiration via ^{14}C heterotrophic uptake of acetate, and microbial community composition via terminal restriction fragment length polymorphism (T-RFLP) analysis. The results demonstrated that moisture regime is a significant factor in decomposition, with high decomposition at wetter sites. Vegetation type also impacted decomposition, as maple leaves were found to decay more similarly across sites, while the breakdown of site-specific vegetation varied more. These findings lack evidence to suggest one variable, moisture or vegetation time, as the driving factor of decomposition. Respiration rates varied greatly between sites and over time. Surprisingly, fungi were found to be a significant contributor to respiration at sites of high moisture, which suggests a need to better incorporate their activity in carbon budgets. Microbial communities were unique at each site and shifts were observed over time for both the bacterial and fungal populations. Changes in community structure were well correlated with changes in OM quality and quantity, though specific relationships varied by site. Future work determining functional groups and taxa of these microbial assemblages would provide a deeper knowledge of the role of these communities on decomposition processes. A better understanding of how differences in soil moisture impact decomposition rates will provide greater insight on the

carbon sequestered or released from a habitat, which may be particularly important with global climate change. Although sites of high moisture exhibited accelerated decomposition, moisture alone may not be the driving factor. In turn, variables associated with high moisture, such as increased nutrients, should be further researched as they may actually be behind the increase in decomposition.

Introduction

The decomposition of plant litter serves an important function in the nutrient cycling of ecosystems by disintegrating dead organic matter (OM) and mineralizing complex organic compounds into simple inorganic forms (Barbhuiya et al., 2008). There are a number of physical, chemical, and biological processes responsible for the break down of plant litter, including leaching, mechanical fragmentation, microbial decomposition, and consumption by invertebrate shredders (Schlieff & Mutz, 2009). Of the aforementioned mechanisms, the greatest loss of organic matter is through heterotrophic breakdown by microorganisms (Baldy et al., 1995). Bacteria and fungi are the main decomposers, though there is no consensus as to which of these groups is dominant (Buesing & Gessner, 2006) or how the abundance or distribution of the two may vary over time (Gutknecht et al., 2006). Moreover, there is a lack of understanding as to how environmental parameters such as moisture and temperature interact to control temporal patterns of the microbial community or whether differences in plant litter quality influence the abundance and function of each population.

Historically, bacteria have been considered to be the main decomposers due to their ubiquitous distribution and high abundance (Gaur et al., 1992; Lopez et al., 1977; Newell, 1965). However, some have proposed that fungi are dominant (Gessner, 1977; Gulis &

Suberkropp, 2003; Kuehn et al., 2000; May, 1974) due to their high measured biomass on decomposing OM (Hieber & Gessner, 2002) and their ability to break down more recalcitrant plant compounds such as lignin using specialized enzymes and hyphal penetration (de Boer et al., 2005; Van Ryckegem et al., 2007).

Previous work on litter decomposition has shown the dominant microbial group may change over time based on season (Habekost et al., 2008) or with changes in organic matter quality (Fioretto et al., 2007). It has been suggested that the relative importance of bacteria versus fungi changes in a predictable manner over time, and may follow an orderly successional pattern. For example, Dilly & Imler (1998) proposed that decomposition occurs in two phases: the first phase consists mainly of bacterial colonization, with few fungi present, while saprophytic fungi dominate in the second stage. These findings were supported by Poll et al. (2008) who found bacteria to dominate the mineralization of easily available substrate followed by fungal depolymerization of more complex litter compounds. Other studies contradict these findings, arguing that fungi will arise first and begin the decomposition process, followed by bacteria (Suberkropp & Weyers, 1996). Recent work of Gaudes et al. (2009) supports the later; when measuring decomposition of leaf litter in a Mediterranean stream, fungal degradation dominated for the first 70 days. Baldy et al. (1995) similarly found fungi to be important in initial litter breakdown in river habitats, and bacterial contributions were small until more advanced stages of decomposition.

One possible explanation for these conflicting findings may be environmental variability. A number of abiotic factors such as temperature, pH, and moisture may

influence microbial communities, which will in turn impact decomposition. The impact of moisture on decomposition is considered to be especially significant (Donnelly et al., 1990), and an optimum moisture range is needed for the greatest decomposition (Chen et al., 2000). Water and oxygen availability are important factors governing the influence of moisture on decomposition. Microbes need water to carry out many metabolic processes, so in habitats of low water availability, decomposition is generally expected to be lower (Reddy et al., 2009). Similarly, in highly saturated habitats, decomposition is expected to be lower; in this case, slow diffusion of oxygen results in anaerobic conditions, which are thought to decrease microbial activity (Dyckmans et al., 2006) and result in less energy-efficient anaerobic pathways (Borglin et al., 2004). For example, decomposition in salt marshes is positively influenced by the flooding process with decomposition increasing with flooding (Halupa & Howes, 1995). Similarly, in forest environments, Schimel et al. (1999) found that soil moisture was a driving factor in OM loss. In considering the microbial agents of decomposition, Su et al. (2007) found bacterial abundance and productivity was higher in inundated wetland sites, while fungal biomass and productivity was greatest at exposed (drier) sites. By controlling decomposition rates and microbial activity, differences in soil moisture can, in turn, affect the carbon sequestered or released from an ecosystem. Increased soil moisture has been found to accelerate OM decay (Bontti et al., 2009; Gonzalez et al., 2008) and has also demonstrated higher microbial respiration rates, releasing more carbon to the environment (DeBusk & Reddy, 2003; Valentini et al., 2008). However, these studies were all conducted in terrestrial sites without the consideration of saturated conditions when anoxia may become an important limitation.

In addition to moisture and environmental controls, some have suggested that the relative importance of bacteria versus fungi in decomposition may covary depending on type of OM substrate that is available (Buesing et al., 2009; Castanho & de Oliveira, 2008; DeBusk & Reddy, 2003). For example, substrate quality, most typically assessed as the carbon to nitrogen ratio (C:N) of the OM, greatly influences the decomposition process (Gutknecht et al., 2006), and may differ between habitats with diverse vegetation. Different litter types show different microbial successional patterns and rates of OM decay (Aneja et al., 2006), and litter with lower C:N is preferentially consumed by microbial communities (Fioretto et al., 2007). Although environmental factors are important in directing the decomposition process, the influences of substrate type and quality should still be considered.

Overall, the relative importance of fungi versus bacteria in the decomposition of organic matter is poorly understood, and it is unclear how the abundance and function of each group may be influenced by environmental variability, site differences, and temporal trends. This research addresses the effects of soil moisture on OM decomposition, microbial respiration, and microbial community composition in three habitats with distinctly different moisture regimes in an effort to better understand how bacterial and fungal succession proceeds during long-term decomposition. Furthermore, decomposition rates and various measures of OM quality were considered in order to account for the effect of litter type. Together these results will enhance the understanding of the microbial interactions and environmental parameters governing OM decomposition and the relative importance of bacteria and fungi on this process.

Methods

Study site

This study was conducted at Virginia Commonwealth University's Walter and Inger Rice Center for Environmental Life Sciences, located twenty-five miles southeast of Richmond, Virginia. The Rice Center is a 138 ha complex of wetland, terrestrial, and aquatic habitats adjacent to the James River. Three sites within Rice were chosen to represent differing moisture regimes in which to study decomposition. The sites were designated as: (1) upland, (2) marsh, and (3) swamp (Appendix 3). The "upland" site had relatively dry soil and deciduous hardwood vegetation characteristic of terrestrial habitats in the mid-Atlantic. The dominant plant species were: *Fagus grandifolia*, *Quercus alba*, and *Quercus rubra*. In contrast, the vegetation at the "marsh" site was primarily comprised of a variety of graminoid species and *Typha angustifolia*. The soil moisture was moderate and standing water was observed on the surface during times of high precipitation. The "swamp" site had highly saturated soil and underwent periods of flooding, and the vegetation consisted of ferns, graminoid species, *Taxodium distichum*, and deciduous hardwood trees such as *Nyssa sylvatica*, which created a dense canopy.

Within each of the three sites, two replicate 10 m x 10 m sub-sites were established approximately 10 m apart.

Soil sampling

Surface soil samples were collected each month (March 2008-May 2009) as a means of monitoring soil pH, redox, and moisture across sites. Each time, three soil samples (~100 grams each) were collected from each sub-site and transported to the lab on ice. Prior to sampling, the surface OM layer was pushed aside and leaf litter was removed. Redox potential and pH were measured using a Hanna Combo pH and ORP probe (QA Supplies Norfolk, VA), and soil moisture content was analyzed gravimetrically ($100 \pm 5^\circ\text{C}$ for 72 hrs). Any remaining sample was then stored at -20°C for later analysis of bulk soil properties.

At the end of the study, the remaining frozen samples were pooled and homogenized to generate a single aggregate soil sample for each sub-site. From the aggregate samples, soil organic matter content, cation availability, and texture were measured (A&L Laboratories, INC., Richmond, VA).

Litterbag Construction and Vegetation Collection

This decomposition study implemented the litterbag technique (Bradford et al., 2002). Litterbags were constructed from 0.5 mm Nitex mesh (Wildlife Supply Company, Buffalo, NY, 24-C60) with the dimensions of 15 cm x 15 cm, and assembled using

polyester thread. The 0.5 mm mesh size was chosen to maximize microbial colonization while minimizing the entry of detritivores and possible loss of litter due to fragmentation.

Two types of plant litter were used in this experiment: (i) site-specific vegetation and (ii) red maple leaves. In November 2007, senesced site-specific litter was collected from each habitat, and senesced red maple leaves were collected from a single nearby tree. The site-specific vegetation at each site differed: the upland vegetation bags were filled primarily with *Fagus grandifolia*, *Quercus alba*, and *Quercus rubra* leaves, the marsh bags contained a mix of graminoid species and *Typha angustifolia*, and the swamp bags included a mix of *Nyssa sylvatica* leaves, ferns, graminoid species, and *Taxodium distichum* leaves. The collected vegetation was allowed to air dry for 14 days. After drying, the site-specific litter was cut into small pieces and homogenized in plastic bags. Four types of litterbags were then constructed using either: (i) red maple, (ii) upland vegetation, (iii) marsh vegetation, or (iv) swamp vegetation. Each bag was filled with ~ 5 g of the appropriate dry litter and sewn shut.

Experimental design

This study was designed to simulate natural vegetation senescent cycles over the growing season. Following senescence in November 2007, the completed litterbags were tied to flags with monofilament and left at their appropriate site; the site-specific bags were placed at the sites from which they were collected, while maple bags were placed at all sites. Immediately, four maple bags from each sub-site and two site-specific bags from each sub-site were collected (t=0 months). These bags were analyzed for moisture content

and percent OM (ash-free dry mass (AFDM)). These values were used to standardize the initial moisture content and initial organic matter content of all the litterbags.

The remaining bags were collected after 3, 6, 10, and 16 months of incubation in the field. On each collection date, typically four bags of each vegetation type were collected from each sub-site, for a total of 48 bags at each sampling. Upon collection, the samples were placed in ziplock bags and brought back to the lab. External soil and debris were gently wiped from the bags, and the bags were weighed. The bags were then cut open, and the contents were homogenized by grinding. Within 24 hrs, samples were analyzed for mass remaining, organic matter content, and microbial respiration. The remainder of the samples were stored (-80°C) for genetic analysis of the microbial communities, dissolved organic carbon (DOC) leaching, and carbon and nitrogen content.

OM loss

From the collected bags, the percent OM remaining was calculated as a means of monitoring decomposition across the three moisture regimes. Using ~1 g subsamples of ground litter, moisture content was determined gravimetrically (50± 5°C for 48 hrs), and the AFDM of the sample was determined following combustion (500± 5°C for 4 hrs).

C:N

The total carbon and nitrogen content of the litter was determined for both the site-specific vegetation and the red maple substrate using 5-10 mg subsamples from each bag. Subsamples were acidified (10% HCl), oven dried (48 hrs at 55 ± 5°C), and then ground

using a mortar and pestle. The ground litter was then analyzed using a Perkin Elmer CHNS/O Analyzer (Waltman, MA), and C:N ratios were calculated.

DOC Release

Dissolved organic carbon (DOC) release associated with leaching of both the site-specific vegetation and the red maple leaf was determined using the approach outlined in Su et al. (2007). A ~ 0.25 g subsample of each recovered sample was placed in a glass vial with 15 ml of deionized water. Vials were then covered with foil, to protect the contents from photodegradation, and placed on a shaker table for 24 hrs at 44 rpm. Once removed from the shaker, 10 ml of the liquid was syringe filtered through a combusted (450°C, 5 hrs) 0.7 µm pore-size filter (Whatman, GF/F) and then diluted via addition of 15 ml of deionized water. Samples were preserved by adding 150 µl of concentrated HCl and analyzed using a Shimadzu TOC Analyzer (Torrence, CA).

Microbial Respiration

Rates of microbial respiration associated with the heterotrophic breakdown of the site-specific vegetation were assessed by examining the total production of ¹⁴C-labeled CO₂ following incubation with ¹⁴C-radiolabeled acetate (Franklin & Mills, 2006). The separate contribution of bacteria and fungi to the total respiration rate was determined via selective inhibition using the antimicrobials cycloheximide and streptomycin (Velvis, 1997; Ananyeva et al., 2006). For each sampling event and each sub-site, two of the litterbags containing site-specific vegetation were randomly selected for this analysis.

Litter from each bag was first ground and homogenized and then five 0.25 g portions were weighed and distributed to separate sterile culture flasks (55 ml volume, Corning Inc, Corning, NY, #3055) preloaded with 15 ml of sterile PBS (pH = 7.4). Flasks were then assigned to one of the five treatments: (i) unamended, to measure total respiration; (ii) an antibacterial treatment with 40 mg of streptomycin per g of litter, to measure fungal respiration; (iii) an antifungal treatment with 80 mg cyclohexamide per g of litter, to measure bacterial respiration; (iv) a combined antibacterial and antifungal treatment with 40 mg of streptomycin and 80 mg cyclohexamide per g of litter; and (v) and a killed control treatment with 1 ml of 2N H₂SO₄ added to stop all microbial activity. Flasks were incubated ~ 2.5 hrs, to allow sufficient time for the antimicrobials to be effective, and then each was spiked and 0.5 μCi of ¹⁴C-acetate (NEN Life Sciences Products, Boston, MA). Following another 2.5 hrs incubation, CO₂ from the headspace of the flask was trapped using phenethylamine, and the amount of ¹⁴CO₂ produced was measured using a Beckman LS 6500 scintillation counter. Respiration rates were then calculated as the amount of ¹⁴CO₂ generated per hour by each microbial group after subtracting the quantity generated in the H₂SO₄-killed controls.

Whole-community DNA extraction and quantification

For each sampling event, microbial community DNA was extracted from the site-specific vegetation, using two litterbags from each sub-site. Extractions were taken from 0.25 g subsamples using the MoBio Labs Power Soil DNA kit (Carlsbad, CA), and 10 μl aliquots of each DNA extract were run on a 1.5% agarose gel to validate size and integrity

of the DNA. DNA concentration was determined using a Nanodrop ND-1000 (Thermo Scientific, Willmington DE), and DNA was stored at -20°C until analysis via T-RFLP.

Microbial community composition using T-RFLP

Microbial community structure and composition were analyzed using Terminal Restriction Fragment Length Polymorphism (T-RFLP) DNA fingerprinting of whole-community DNA extracts (Liu et al., 1997). The bacteria present in the community were targeted using a 6FAM (fluorescently labeled) forward primer, Bac 27F (5'-AGAGTTTGATCCTGGCTCAG-3'), and a reverse primer (unlabeled), Bac 1492R (5'-GGTACCTTGTTACGACTT-3'), which amplify the 16s subunit of the ribosomal RNA gene (Hayashi et al., 2002). For the fungal community, a TET (fluorescently labeled) forward primer, ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3'), was utilized along with a reverse primer (unlabeled), ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). These primers amplify the intergenic transcribed spacer region (ITS) of ribosomal DNA (Hanson et al., 2008). All primers were obtained from Integrated DNA Technologies, Coralville, Iowa.

The bacterial community PCR was conducted in 50 μl reactions that contained between 2.5 and 10 ng of template DNA, 10.0 mM Tris HCl (pH 8.3), 50.0 mM KCl, 3.0 mM MgCl₂, 200 μM of each dNTP, 0.1 μM of each primer, 20 mg BSA (bovine serum albumin; Hoffman-La Roche Inc., Nutley, NJ), and 2.5 units of AmpliTaq DNA polymerase (reagents obtained from Applied Biosystems, Foster City, California). PCR amplification used the following conditions: initial denaturation at 95°C for 3 minutes

followed by 27 cycles of 45 seconds at 94°C, 1 minute at 57°C, and 2 minutes at 72°C, and a final elongation step of 72°C for 7 minutes (PTC-100 Thermal Controller, MJ Research, Inc. Waltham, Massachusetts). The fungal community PCR followed identical reaction conditions but with a total cycle number of 32 and an annealing temperature of 52°C. Successful amplification was confirmed by agarose gel electrophoresis of 10 µl aliquots of the PCR product on a 1.5% gel, followed by ethidium bromide (EtBr) staining.

Each set of PCR products was purified using the MiniElute PCR Purification Kit (Qiagen, Valencia, California). Bacterial PCR products were then digested using the restriction enzyme Msp, while the fungal PCR products were digested using the restriction enzyme Hha1. Restriction digests were carried out in 10 µl reactions made of 1 µl of enzyme (20,000 U µl⁻¹), 1 µl of Buffer 4 (10X), 7 µl of the cleaned PCR product, and 1 µl water. All enzymes and buffers were obtained from New England Biolabs Inc. (Ipswich, MA). Digests were conducted at 37°C for 6 hrs, followed by deactivation at 70°C for 6 min.

Once the digests were complete, the PCR products were cleaned again with the MiniElute PCR Purification Kit (Qiagen, Valencia, California) and followed by capillary electrophoresis on a MegaBACE 1500 Series Sequencer (Amersham Biosciences, Piscataway, New Jersey). Electrophoresis used 8 µl of each purified digest and 0.25 µl of MM 400 size standard (BioVentures, Inc., Murfreesboro, Tennessee). Samples were injected at 3 kV for 100 seconds and ran for 100 min at 10 kV. The data generated by the fragment analysis of the digested PCR product were analyzed using MegaBACE Genetic

Profiler Software Suite v2.2, specifically the Fragment Profiler program (Amersham Biosciences).

Data analysis

Site and vegetation effects on litter decomposition: For each sampling, the importance of site (upland, marsh, swamp) and vegetation type (site-specific or maple) on a given variable was evaluated using a two-factor analysis of variance (ANOVA). Whenever a significant site-by-vegetation interaction was detected ($\alpha=0.05$), individual one-factor ANOVA's were used to test either factor separately, using a Tukey analysis for post-hoc comparisons. Separate analyses were used to examine the amount of decomposition (percent OM remaining) and the quality of the resultant OM (assessed via DOC release or C:N). All data analyses were performed using the SPSS 16.0 statistical software (Chicago, IL).

Bacterial and fungal respiration rates: A single-factor ANOVA was used to determine differences across sites in respiration rates of each microbial group. Analysis was conducted for both bacteria and fungi separately at each individual sampling period ($\alpha=0.05$) and followed by a Tukey's post hoc analysis. In addition, a two-tailed t-test was used to compare the bacterial and fungal respiration at each site for each sampling period ($\alpha=0.05$).

Microbial community composition: Analysis of microbial community composition was carried out separately on the bacterial and fungal datasets as follows. First, T-RFLP electropherograms were analyzed using the MegaBACE Genetic Profiler Software Suite

v2., which recorded the presence or absence of each peak (a putative bacterial or fungal species) in each sample as part of a binary data matrix. These data were then analyzed via non-metric multidimensional scaling (NDMS) with Jacquard's distance coefficient using the PAST (PAleontological STatistics) program (Hammer and Harper, 2001). Plots of the first two dimensions were produced as a means of visualizing the differences among communities based on both site and sampling time. An analysis of similarities (ANOSIM) was used to test statistically whether there were any significant differences between communities from each site or over time using the Jacquard coefficient and a Bonferroni corrected α of 0.05.

Correlation analysis: A Pearson's correlation analysis was conducted to determine if relationships were present among many of the measured variables at each site. Prior to the correlation analysis, all data was first tested for normal distribution by a Shapiro-Wilk test using JMP 8.0. Each variable was normally distributed except for DOC, which could not be transformed to meet a normal distribution due to the fact that all t=16 samples measured below the detection limit. To remedy this, DOC data for t=16 was removed from the correlation analysis. A Bonferroni corrected α of 0.005 was applied for the multiple comparisons.

Results

Site Characteristics

Soils at all three sites were dominated by silt and sand, with low clay content (Table 1). Organic matter was also low and did not differ across sites. Soil cations (phosphorus, potassium, magnesium, and calcium) varied considerably. Calcium was the most abundant cation for all sites and greatest in the swamp. Phosphorus was the most limited cation for all sites and lowest in the marsh site. The CEC (cation exchange capacity) increased with moisture content from the upland site to the swamp site (Table 1).

In addition, monthly soil sampling demonstrated that all three sites were distinct with regards to pH (Figure 1A), redox potential (Figure 1B), and gravimetric moisture content (Figure 1C) during the course of this study. The swamp site had the highest pH (avg.=6.2), lowest redox potential (avg.=3 mV), and the largest gravimetric moisture content (avg.=299%; Table 1). The pH did not differ between the other two sites (marsh avg.=4.7, upland avg.=4.7), though they were distinct with regard to redox potential and moisture content. Redox was consistently higher at the upland site, where moisture content was lower (avg. moisture upland=40%, marsh avg. moisture=109%). In general, moisture content showed similar monthly and annual trends across the three sites (Figure 1C).

Percent OM remaining

A single-factor ANOVA indicated the initial (t=0) percent organic matter was not significantly different across the vegetation types (p=0.931) prior to field incubation. For all study sites and vegetation types, the greatest OM decomposition occurred during the summer months (June 2008 to Oct. 2008), between collection dates t=6 and t=10 (Table 2, Figure 2). The maple leaves decomposed similarly at all sites (Figure 2A), but breakdown of site-specific vegetation depended on location. The greatest amount of decomposition was at the marsh site and the lowest was seen at the upland (Figure 2B).

A two-factor ANOVA determined that the main factor affecting the amount of OM remaining after three months of incubation was site (p=0.007). The % OM remaining was highest at the upland site (94%) and lowest at the swamp site (87%) with the marsh site being similar to both (90%). At all other sampling times, the two-factor ANOVA revealed a significant interaction between site and vegetation type (Table 3). Vegetation type was most important at the upland site (t=6, p<0.001; t=10, p<0.001; t=16, p=0.101), with higher decomposition for the maple leaves relative to the site-specific vegetation (Table 4). In contrast, there were few significant differences in % OM remaining between the two vegetation types when incubated at either the marsh or swamp sites. For the former, maple leaves had significantly less OM remaining after 10 months in the field (p<0.001) while the swamp showed no significant difference (p=0.197; Table 4).

When considering differences in decomposition, there is a clear effect of site, regardless of substrate type. At t=6, the % OM remaining for both maple (F=4.29, p=0.027) and site-specific vegetation (F=9.32, p=0.002) was significantly different across

the moisture gradient (Table 4). A post hoc analysis demonstrated that maple leaves had decomposed the most at the upland site, while the site-specific vegetation decomposed the least at the upland site (Figure 2). Both vegetation types showed significantly different % OM remaining for t=10 (maple: $F=4.40$, $p=0.025$; site-specific: $F=15.46$, $p=0.001$). A post hoc analysis determined the swamp and marsh % OM remaining to be significantly different for the maple leaves, with the swamp having the greater % OM remaining. In comparison, the upland had the greatest % OM remaining for the site-specific vegetation. The site-specific vegetation % OM remaining at t=16 was significantly different across all sites ($F=4.40$, $p=0.046$) and, from the post hoc analysis, the marsh site had the least % OM remaining (Figure 2).

C:N

The C:N of the undecomposed litter was higher in the maple leaves (average=78) relative to site-specific vegetation, which was significantly lower (upland=65; marsh=47; swamp=28) as determined by a single-factor ANOVA ($p<0.001$). As decomposition progressed, C:N decreased for both types of litter; however the magnitude of the effect depended on the incubation site (Figure 3).

At the first sampling (t=3), a two-factor ANOVA demonstrated that the C:N of the litter was significantly different based on site ($F=149.0$, $p<0.001$) and vegetation type ($F=17.3$, $p<0.001$), but there was no significant interaction between the two factors ($p=0.169$). The C:N was always higher for the maple when compared to the site-specific vegetation of each site. For both vegetation types, the incubation site had an effect on C:N,

with wetter sites having significantly lower ratios. For all of the later samplings, a significant site-by-vegetation interaction effect was detected using the two-factor ANOVA (t=6, 10, 16; Table 3). At t=6, the C:N for the different vegetation types were significantly different at every site (Table 4), with the maple leaves having a higher ratio. This was also seen for the upland site, at t=10 (p=0.005) and the marsh site at t=10 (p=0.005) and t=16 (p=0.003).

When the effect of site alone was considered, significant differences were seen for both vegetation types (Table 4). A consistent trend was seen across sites for the maple leaves at later sampling periods (t=6, 10, 16) with C:N decreasing with moisture (Figure 3A) from the upland site to the swamp site (all, p<0.001). This same trend was seen for the site-specific vegetation. For t=10 (p<0.001) and t=16 (p<0.001), C:N of site-specific vegetation was further different by site, with the upland having the lowest ratio while the marsh and swamp were similar.

DOC Release

A single-factor ANOVA demonstrated the initial (t=0) lab extractable DOC was highest for the maples leaves (56.7 mg g⁻¹ OM) and was not significantly different among the site-specific vegetation (p<0.001). For the first several months of the study (t=3, 6), the two-way ANOVA did not reveal any significant interactions between site and vegetation type. When the factors were considered separately, site effects were insignificant (t=0 and t=3, p>0.10) and differences in DOC were due only to vegetation (Table 3). In both cases, maple leaves contained a greater amount of lab extractable DOC.

In contrast, at $t=6$, the incubation site had a significant effect on the laboratory DOC release ($F=14.41$, $p<0.001$), while vegetation type was not a significant influence. A post hoc analysis showed that the lab extractable DOC released from upland samples was the greatest (Figure 4).

At $t=10$, the interaction of vegetation type and site was significant ($p=0.011$; Table 3). At the upland site, DOC release was significantly different between vegetation types ($F=20.49$, $p<0.001$) with the maple leaves releasing a greater amount (Figure 4, Table 4). For both vegetation types, DOC release was significantly different across sites (Table 4), with the upland samples releasing the most DOC in the laboratory (Figure 4). At the final sampling ($t=16$) laboratory DOC release was below the detection limit for all samples from all sites (Figure 4).

Microbial Respiration

Total microbial respiration rates varied depending on sampling date (Figure 5). In general, respiration rates were higher for the swamp site and lowest for the upland. Rates measured at the swamp changed greatly with time, peaking during the early incubations ($t=3$ and 6) at 5 to 6 times the later values. For the marsh, respiration rates were quite low at $t=3$, and instead higher at latter times. Values for the upland changed little with time and always had lower rates relative to the other two sites.

For each sampling time, bacterial respiration rates were found to be significantly different across sites using a single-factor ANOVA (Table 5). Initially ($t=3$), the swamp site had the highest rate of bacterial respiration (Figure 5A). At intermediate sampling

periods, $t=6$ and $t=10$, the marsh and swamp sites' rates of respiration were greater than the upland site's rates but not distinct from one another. Whereas at $t=16$, the marsh site had the highest rate of bacterial respiration. As with bacteria, fungal respiration rates were significantly different across sites for each individual sampling time (Figure 6B, Table 6). In general, rates were highest for the swamp, while rates at the marsh and upland sites were not significantly different. The only exception to this was $t=10$, when rates in the marsh increased to the same level as the swamp. The only consistent temporal trend was for the swamp site, where respiration rates at later sampling periods ($t=10$ and 16) were considerably lower than the earlier sampling periods ($t=3$ and 6).

For each site and each sampling period, the fraction of total respiration attributable to each microbial group was calculated (Figure 7). In the marsh, most of the respiration was due to the bacterial portion of the community for all times sampled. In the upland, fungal respiration dominated, except for the final sampling date ($t=16$). No clear pattern was evident for the marsh site, where bacterial and fungal respiration each dominated at different times. A two-tailed t-test was used to compare bacterial and fungal respiration rates for each site at each time (Table 7). Initially ($t=3$), rates at the upland site were not significantly different between the two groups. With time, the upland respiration was dominated by fungi at $t=6$ ($p=0.008$) and $t=10$ ($p=0.026$), but at the final sampling period ($t=16$), bacterial respiration peaked and surpassed fungal respiration ($p=0.050$). Similar to the upland site, the marsh site's initial ($t=3$) fungal and bacterial respiration rates were not significantly different. For the remainder of the sampling periods, bacterial respiration rates were elevated relative to fungal ($t=6$, $p<0.001$; $t=10$, $p=0.043$; $t=16$, $p<0.001$).

Lastly, bacterial and fungal respiration at the swamp site showed a contrasting pattern with alternating peaks in bacterial and fungal respiration at each sampling period. Initially (t=3), bacterial respiration was much higher than fungal respiration (p=0.035) and was also higher at t=10 (p=0.05), whereas for the sampling periods, microbial respiration was not significantly different between the two groups.

Microbial Community Composition

To evaluate the individual effect site has on the microbial communities, the results from all sampling periods were combined for each microbial group at each site and analyzed via NMDS. Even when temporal effects were pooled, there was clear difference in microbial community composition between the different sites. For the bacterial communities, all three sites separated, with the swamp site the most distinct (Figure 8A). For the fungi, the swamp community was also the most unique group, while the marsh and upland were not significantly different (Figure 8B).

Similar plots were made to look for major trends associated with time, regardless of the incubation site. Bacterial communities were grouped together for t=3, t=6, and t=10, but the t=16 community was much different than the others (Figure 9A). Fungal communities were more influenced by incubation time, with all groups distinct (Figure 9B).

For each individual sampling period, both microbial groups' community composition of each site was found (Figure 10). Both microbial groups exhibited shifts in community composition at each site during the field incubation. For each site, an

ANOSIM was used to test for significant differences over time ($\alpha=0.05$). At the upland site, little change was seen in the bacterial community until a shift at $t=16$, whereas the fungi community demonstrated shifts at each sampling period. At the swamp site, an opposite pattern was seen with the bacterial community shifting at each sampling period while the fungal community exhibited little change until $t=16$. Lastly, the marsh site showed continual shifts throughout the experiment for both microbial groups.

Correlations among Tested Variables

The Pearson's correlation analysis demonstrated several statistically significant relationships at the upland (Table 8), marsh (Table 9), and swamp (Table 10) sites. At all three sites, the amount of DOC leached correlated with the C:N of the litter (r for upland = 0.637, marsh = 0.845, swamp = 0.580) indicating that these two measures of substrate quality were linked. In addition, the amount of substrate (% OM remaining) was correlated with both C:N ($r = 0.481$) and DOC ($r = 0.599$) for the marsh site.

Bacterial and fungal respiration rates were strongly correlated with one another at both the upland ($r = 0.710$) and marsh ($r = 0.861$) sites, but not at the swamp site ($r=8.282$). When respiration rates are compared to the various assessments of substrate quantity and quality, different variables appear to be important at different sites. At the upland site, % OM remaining was negatively correlated with both bacterial respiration ($r=-0.775$, $p<0.005$) and fungal respiration ($r=-0.846$, $p<0.0005$), but neither correlated with C:N or DOC. For the marsh, respiration rates were only correlated with DOC leached (for bacteria: $r=-0.766$, $p=0.004$; for fungi, the correlation was nearly significant: $r=-0.680$,

p=0.01). In the swamp, no significant correlations were detected for fungi, and bacterial respiration rates were correlation with C:N ($r=0.742$, $p<0.001$).

To analyze how these variables were correlated with microbial community structure, the values for the first two NDMS axes were considered to represent either the bacterial or fungal subset of the community. At the swamp site, very few significant relationships were observed. C:N was correlated with both sets of organisms (bacteria axis 1: $r=-0.673$, $p=0.005$; nearly significant for fungi axis 1: $r=-0.581$, $p=0.1$). In addition, the community structure of the two groups was correlated (bacteria axis 1 is correlated with fungi axis 1: $r=0.798$, $p<0.001$).

At the marsh site, fungal community structure was not significantly correlated with any of the other variables. In contrast, bacterial community structure was correlated with several variables including C:N (bacteria axis 2: $r=-0.678$, $p=0.004$), DOC (nearly significant with axis 1: $r=0.679$, $p=0.01$), and % OM remaining (nearly significant with axis 2: $r=-0.597$, $p=0.01$). The only significant correlation at the upland site was between NMDS bacteria axis 1 and bacterial respiration ($r=0.887$, $p<0.001$).

Discussion

Differences in Decomposition by Site and With Time

Decomposition varied both across sites and over the time course of the study with differences in decomposition seen among the vegetation types. Breakdown of site-specific litter was usually less in habitats with lower moisture content (upland site) and generally increased at the wetter sites (marsh and swamp; Figure 2B). Maple decomposition showed no clear moisture effect, suggesting that differences in vegetation type may influence decomposition (Figure 2A). Based on the present findings, there was insufficient evidence to support one variable, moisture or vegetation type, as the principal driver of decomposition. These results also indicate that the decomposition process is potentially controlled by a complex composite of variables, not just one single variable.

Although the results were found inconclusive, and moisture was not named the controlling factor of decomposition, a strong moisture effect was demonstrated. As previously mentioned, the decomposition of the site-specific vegetation increased with moisture, and even though maple decomposition did not demonstrate a significant moisture trend, maple samples did show high decomposition in wet sites (Figure 2). A pattern of OM decomposition increasing with moisture has been found in several studies across a variety of ecosystems including grasslands (Bontti et al., 2009), boreal, temperate, and tropical forests (Gonzalez, et al., 2008), marshes (Halupa & Howes, 1995), and in

controlled laboratory settings (Van Meeteren et al., 2007). The results of the present study were somewhat surprising in that the saturated conditions of the swamp site were originally expected to limit decomposition by promoting anaerobic conditions, but both vegetation types showed significant decomposition at this site. These findings may indicate that oxygen limitation is not as influential as previously thought, or the effect is somehow offset by another variable at the swamp site. Battle & Golladay (2007) studied different wetland types in Georgia and similarly observed that increased moisture and inundation period enhanced OM decomposition, and suggested this was due to nutrient replenishment (such as nitrogen and phosphorus) from flooding conditions stimulating the microbial assemblages. In the present study, the enhanced decomposition at the swamp site may be a result of not only high moisture, but the influx of new nutrients to this system, particularly nitrogen and phosphorus from agricultural activities in the adjacent watershed and the tidal flux into the swamp from the James River. It is also important to note that although the greatest moisture content was demonstrated at the swamp site throughout the study, the site was not permanently inundated and underwent periods of flooding and drawback after high precipitation events. This means the microbial communities likely experienced both aerobic and anaerobic conditions, which would increase decomposition compared to permanently saturated sites (Battle & Golladay, 2001).

In addition to differences in decomposition between sites, decomposition rates varied over time. In particular, more OM breakdown occurred during the summer (June-October 2008) between the t=6 and t=10 collection dates (Table 2). This was expected as

decomposition has been found to increase with temperature (Barbhuiya et al., 2008; Van Meeteren et al., 2007). In colder temperatures, microbial metabolic activity slows, therefore decomposition rates will be lower (Gaudes et al., 2009; Kominkova et al., 2000). The greatest increase in decay during the summer months was observed at the marsh site. This site differs from the other sites in that it lacks canopy cover, which likely increased the soil temperatures of that site, further accelerating microbial activity compared to the upland and marsh sites (Van Meeteren et al., 2007). Moreover, the decreased canopy cover at this site means the litter had greater exposure to solar radiation (UV), which could lead to photodegradation of OM (Austin & Vivanco, 2006; Gallo et al., 2006).

In order to gain a better understanding of how litter quality changed over time and by site, C:N and DOC release of the litter were measured. With time, the C:N of the litter decreased as OM was lost (Figure 2 and 3). This was due to the fact that much of the carbon associated with the organic matter was respired through catabolic pathways and left the system as CO₂ (Kominoski et al., 2009; Wang et al., 2009). In contrast, nitrogen may be preferentially retained and recycled between organic and inorganic forms (Raghubanshi, 2008) resulting in the observed decrease in carbon relative to nitrogen (i.e., lower C:N, Figure 3). In the present study, C:N values of both site-specific vegetation and maple leaves converge on a similar value (20:1-35:1) with slightly elevated ratios at the upland site (Figure 3). This value is near the critical ratio of C:N, 25:1; above this value it is thought that there will be no net nitrogen released from decomposing OM, and below this value excess nitrogen may be excreted from microbes in to the environment (Chapin III et al., 2002). Changes in C:N should be minimal once the critical ratio has been reached; this

was demonstrated over the final 6 month incubation period of this study as changes in the C:N were negligible (Figure 3). With time, C:N differed by site for each vegetation type, as soil moisture increased the C:N decreased. Although soil moisture did not significantly affect the % OM remaining of the maple leaves, it appears to have had an influence on maple leaf C:N.

When measuring DOC release, initially lab extractable DOC was high, but after further incubation, measurements were much lower (Figure 4) as DOC is an easily degradable substrate for microbes, and it is expected to be quickly consumed upon release (Hagedorn & Machwitz, 2007). This was especially pronounced at the sites of higher moisture content, with the litter of these sites leaching less lab extractable DOC suggesting high *in situ* leaching. High moisture environments have been found to allow greater leaching of water extractable carbon, thereby promoting overall carbon loss (Halupa & Howes, 1995). Sites of high precipitation, which in turn would possess high soil moisture, have also displayed increased release of *in situ* DOC (Rees et al., 2006). By the end of the study, all samples' lab extractable DOC was below the detection limit, suggesting all samples' DOC had been released *in situ*. After the 16 month incubation period, due to decreased C:N and DOC release, a decline in decomposition would be expected as the more labile compounds have been preferentially consumed by microorganisms, leaving the more refractory compounds.

Vegetation Effects on Decomposition

Differences in decomposition, in terms of % OM remaining, C:N, and DOC release were seen between the maple leaves and the site-specific vegetation (Figure 2, 3, 4). When comparing the % OM remaining for each litter type at each site, significant differences were found (Table 4), however the differences changed with site and time, and no discernible pattern was established. The maple vegetation typically had lower % OM remaining compared the site-specific vegetation for the upland and swamp sites, while the maple and site-specific vegetation of the marsh site % OM remaining was similar (Figure 2). Maple leaves would be expected to decompose faster due to their high amounts of labile carbon, which are preferentially consumed by microorganisms (Chapin III et al., 2002). The decomposition of the upland site-specific vegetation and maple leaves were expected to be similar, as both substrates are deciduous leaves, but maple generally showed higher decomposition. This could be due to the upland site-specific vegetation's composition mainly consisting of oak leaves, which have been found to decay slower than maple leaves (Mudrick et al., 1994) due to a high presence of tannins in oak leaf chemistry (Loranger et al., 2002). A quite plausible explanation for the high decomposition of maple leaves compared to the site-specific vegetation may be due to the collection process of the senesced litter. Maple leaves were collected immediately following senescence from one tree with minimal field exposure prior to litter bag construction, while the site-specific vegetation, in the form of senesced leaves and grasses, may have been exposed to the environment longer before collection and partially decayed litter was potentially included. Newer litter has higher amounts of labile carbon and is prone to greater amounts of

decomposition, while older litter will decompose slower as the palatable labile carbon has already been consumed (Chapin III et al., 2002). If the site-specific vegetation had more field exposure than the maple leaves prior to collection, the maple leaves would be considered newer litter, possessing significantly more labile carbon, and in turn decompose faster.

The litter quality, reflected by C:N, was lower in the maple leaves than in the site-specific vegetation (based on a higher C:N) and also varied across the three sites (Figure 3). Others have found low C:N to degrade faster (Enríquez et al., 1993), but the present study did not find a clear correlation between C:N and decomposition via % OM remaining. Like moisture, the decomposition of a substrate cannot be solely determined by its quality, suggesting environmental parameters, such as moisture and temperature, may have a significant influence as well (Castanho & de Oliveira, 2008; Rejmankova & Houdkova, 2006; Vanderbilt et al., 2008).

A greater initial amount of lab extractable DOC was released by the maple leaves compared to the site-specific vegetation. Like the % OM remaining, DOC release may have also been impacted by the collection process of the senesced litter. Litter type was initially important in determining DOC release, but with time became negligible. Other work has shown DOC release to be a large contributor to initial mass loss of OM (Hagedorn & Machwitz, 2007). A strong correlation between DOC and C:N was found for all sites suggesting initial leaching alters litter quality, particularly C:N (Hagedorn & Machwitz, 2007).

Temporal and Site Trends in Microbial Respiration

As previously mentioned, most of the loss of OM was due to microbial respiration and. In order to better understand natural microbial dynamics associated with this process, microbial respiration rates were measured for the site-specific vegetation. Respiration rates determined the microbial activity at a point in time by CO₂ release. In general, total respiration was highest at the sites of higher moisture content (Figure 5), suggesting that microbial metabolic activity increases with water availability. Across a phosphorus gradient in the Everglades, (DeBusk & Reddy, 2003) found a similar moisture influence on respiration by measuring *in situ* carbon flux. Similarly, when comparing exposed and an inundated wetland habitats, (Su et al., 2007) observed greater respiration rates at the inundated site based on dissolved oxygen consumption. Studies in forest ecosystems have also had similar conclusions (Fioretto et al., 2007). Although prior research has also shown a trend of increased respiration rates with increased moisture, no single study has been conducted across as large of a moisture gradient as the present research.

Of the respiration assay results, the high respiration rates of the swamp site is of particular interest, and may possibly be a consequence of nutrient loading from the runoff of surrounding upland habitats (DeBusk & Reddy, 2003). The total respiration at t=3 is extremely high at the swamp site compared to all other sites and other sampling periods, and corresponds with greatest soil moisture content (Figure 1C). This suggests that the respiration of the swamp site is not negatively impacted by the anaerobic conditions associated with high moisture content, and moisture may not be the only factor increasing respiration rates.

Bacterial and Fungal Contributions to Respiration

In order to better understand the relative importance of bacteria and fungi to the total respiration rates, selective inhibition was used to determine the contributions of these two microbial groups at each site and at each sampling. Each site showed a unique pattern in respiration rates with respect to the dominant microbial group (fungal versus bacterial respiration contributing to the total microbial respiration). Typically, the upland site showed higher rates of fungal respiration, whereas bacterial respiration dominated the marsh site, and bacterial and fungal respiration rates were essentially equivalent at the swamp site (Figure 7). The high rates of fungal respiration at the upland terrestrial site are consistent with recent findings by de Boer et al. (2005), who posit fungi are responsible for a larger portion of organic carbon breakdown at these locations compared to bacteria due to fungi's affinity for lower moisture levels. Although bacterial respiration was typically greater than fungal respiration at the marsh site, fungal rates were still significant. The high levels of fungal respiration at the wet sites contradicts core findings suggesting a low fungal activity in habitats of high moisture content due to detrimental effects of anaerobic conditions (de Boer et al., 2005; Gaur et al., 1992; Reith et al., 2002) and deficiency in enzymatic function (Benner et al., 1984) while supporting less accepted results proposing fungi as significant aquatic decomposers (Baldy & Gessner, 1997; Kominkova et al., 2000; Kuehn et al., 2000). Fungal contributions to respiration rates of high moisture habitats may be more important than previously thought, though most work to date on moisture effects on fungi have typically examined mycorrhizae (Bauer et al., 2003; Miller & Bever,

1999), which are not thought to be significant OM decomposers. With this knowledge gained from this study, fungal respiration should be considered as an integral player in wetland carbon budgets.

Ideally, microbial respiration would have been accompanied by a measurement of microbial abundance to achieve a respiration rate per cell of each microbial group, bacteria or fungi. Due to the lack of this information, currently, biomass of each microbial group can not be partnered with the rate of microbial respiration. Further, the respiration assay was conducted under aerobic conditions. This may have had a negative impact on anaerobic respiration due to oxygen toxicity, in turn leading to underestimates of total respiration. Despite the potential underestimation of anaerobic respiration, the swamp site, which would conceivably have the greatest anaerobic activity due to low redox values (Figure 1B), typically had the highest rates of respiration.

Environmental Parameters Influencing Microbial Respiration and Community

Structure

In addition to observing the changes in bacterial and fungal respiration over the course of the study, variations in the composition of the community of the site-specific litter were also considered. Past research has found microbial communities to be influenced by site; this has been demonstrated in salt marsh habitats (Buesing et al., 2009) as well as stream environments (Harrop et al., 2009). Habitat characteristics, particularly temperature (Buesing et al., 2009) has also been found to impact the community, while others have determined the community to be controlled by stage of decomposition and

temporal variation (Buchan et al., 2003). Drenovsky et al. (2004) found differences in both bacterial and fungal community composition were driven by the different organic matter inputs, as well as water availability. In the present study, distinct bacterial and fungal communities were established for each site (Figure 8), suggesting site had a strong overall influence on microbial community composition. Shifts in both microbial groups were demonstrated at each site over the course of the study (Figure 10).

To better understand the relationship between the microbial communities and their environment along with the effects they might have on one another, correlations among the study measurements were further evaluated. The upland and marsh site both demonstrated positive correlations between bacterial and fungal respiration, suggesting that both groups are impacted by a similar variable or that possibly the microbial groups have a synergistic effect on one another. A synergistic relationship could be due to the microbial groups' provision of growth factors for one another, a simultaneous action of removing inhibitory compounds, or functional enzymes only acting when bacteria and fungi are together (Bengtsson, 1992). At the swamp site, no correlation was found between bacterial and fungal respiration; this may indicate that bacterial and fungal respiration is driven independent of one another.

Both the bacterial and fungal respiration of the upland site correlated with the % OM remaining; fungal respiration was greatest over the first few samplings when the % OM remaining was still high. Also, the fungal community shifted throughout the experiment (Figure 10); these shifts may be associated with changes in OM quality (Groffman et al., 1996). At $t=16$, there was a drop in % OM remaining, a shift from fungi

to bacteria as the main contributor to microbial respiration, and the bacterial community composition shifted there as well. The microbial community shifts were not due to a lack of OM availability, as there was an abundance of OM available (Figure 2), instead these results suggest that moisture may be the parameter impacting community composition at this site. Williams & Rice (2007) found water availability to be important in determining bacterial and fungal communities by imposing both direct (i.e., water potential) and indirect (i.e., leaching of carbon from plant litter) changes. The low moisture of the upland site may inhibit the release of DOC, which is readily taken up by bacteria (de Boer et al., 2005), causing bacteria to wait for availability of simpler OM compounds by fungal breakdown and fungal exudates, which has also been found suitable for bacterial consumption (de Boer et al., 2005).

At the marsh site, bacterial and fungal respiration rates were not correlated with % OM remaining, but were instead correlated with DOC release. Prior research (Docherty et al., 2006) has demonstrated shifts in bacterial community based on dissolved organic matter (DOM) form and quality, particularly that of DOC. Both microbial groups showed continual community shifts over the course of the study as litter quality was altered, suggesting these changes influenced the observed community shifts (Figure 3 and 10). At the marsh site, the litter quality by DOC release may be what is causing these community shifts as each group moves as DOC release decreases.

At the swamp site, bacterial respiration correlated with both DOC release and C:N, while fungal respiration did not. Bacteria community composition also correlated with C:N, implying that the changes in litter quality with increased decomposition impacted the

bacterial community composition (Buchan et al., 2003). The bacteria community shifted continually, exhibiting an association with changes in OM quality. Flooded conditions have been found to have decreased fungal communities and increased bacterial communities (Drenovsky et al., 2004), but the cause of the trend has not been fully explained. The fungal community only shifted at t=16, and was possibly impacted by the low oxygen levels associated with the high soil moisture of the swamp site, causing the fungal community to display minimal changes.

Changes in OM quality and moisture appear to be strong influences on the changes in microbial community composition. Moisture influenced in two different ways; the upland site was influenced by moisture via water availability, which in turn affected microbial processes and OM composition, while the communities of the swamp site, particularly fungi, were impacted by low oxygen levels associated with high moisture content. The changes in OM quality, particularly DOC release, influenced the marsh microbial communities. Consequently, the factors driving community composition, OM quality and moisture, may be responsible for the divergent directions seen in the community shifts, as the upland and swamp communities moved in one direction, while the marsh community moved in the opposite. Based on these findings it can be proposed, that in the future, the directions of shifts in community composition may be predicted by the driving influences.

Conclusions

Based on the results of this research, the release of carbon from habitats will possibly increase with soil moisture due to increases in decomposition and microbial respiration. This knowledge could contribute to a better understanding of the fate of carbon in ecosystems with predicted climate change and accelerations in the hydrologic cycle. The importance of substrate quality on decomposition gave no clear results, but should still be taken into account as further investigation is needed. The results of the respiration assays gave a better understanding of the main players in decomposition with fungal contributions supporting more controversial findings, due to significant fungal respiration in wet habitats. It is important that fungal contributions are included in the carbon budget, particularly in wetland carbon cycling. The impact of tree canopy on decomposition was a variable not taken into initial consideration, but may also be an important environmental factor affecting microbial activity and OM loss independently by photodegradation. In the future, the implementation of quantitative polymerase chain reaction (qPCR) will give much greater insight into the abundance of the microbial assemblages in habitats, which in turn will give more accuracy to the source of respiration as well as the assimilation efficiencies of these two groups. Site was found as a driving factor of microbial community composition, and a potential was demonstrated for the prediction of community shift direction based on the dominant influence on community shifts. Also, by looking at taxa and functional group much insight may be gained about the dominant microbial assemblages present. Lastly, it should be noted that decomposition depends on many aspects of a habitat that interact with one another; there is no one

variable controlling this process. In this study, although sites of high moisture demonstrated high decomposition, moisture can not be assumed to be the driving factor. Other environmental parameters associated with high moisture should be further evaluated independently in order to gain a better understanding of their role in this important biological process.

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APPENDIX A: Tables

Table 1. Soil characteristics for each study site (mean of sub-sites).

Site	pH ^a	Redox ^a (mV)	Moisture Content ^a (%)	%OM	Soil Textural Class	Soil Texture (%)			P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	C.E.C. ^b (meq/100g)
						Sand	Silt	Clay					
Upland	4.7	382	40	5.5	Loam	45	39	16	36.0	108.0	55.0	165	3.70
Marsh	4.7	236	109	3.0	Silt Loam/Loam	30	50	20	12.5	34.5	40.0	295	5.30
Swamp	6.2	3	299	4.4	Silt Loam	27	56	17	36.5	56.5	102.5	800	7.00

^aOverall averages from monthly monitoring (see methods).

^bCation exchange capacity

Table 2. Percent OM lost per week during the prior incubation interval.

Collection period	Incubation	Maple Leaves			Site-Specific Vegetation		
		Upland	Marsh	Swamp	Upland	Marsh	Swamp
t=3	11/07-2/08	0.1	0.4	0.6	0.0	0.4	0.4
t=6	2/08-6/08	1.1	0.3	0.0	0.0	0.2	0.4
t=10	6/08-10/08	0.6	1.0	0.6	0.6	1.7	0.7
t=16	10/08-4/09	0.2	0.3	0.8	0.3	0.0	0.0

Table 3. Results (p-values) of two-factor ANOVA comparing Site and Substrate. If interactions were significant ($p \leq 0.05$), single factor ANOVA's were conducted (Table 4), and individual factor effects are not reported here.

	% OM	C:N	DOC
3 months			
-Site x vegetation	0.124	0.169	0.967
-Site	0.007*	<0.001*	0.362
-Vegetation	0.539	<0.001*	0.027*
6 months			
-Site x vegetation	<0.001*	0.015*	0.121
-Site	-	-	<0.001*
-Vegetation	-	-	0.118
10 months			
-Site x vegetation	<0.001*	0.003*	0.011*
-Site	-	-	-
-Vegetation	-	-	-
16 months			
-Site x vegetation	<0.001*	0.010*	Bd
-Site	-	-	-
-Vegetation	-	-	-

*indicates statistical significance ($p < 0.05$)

Bd indicates all measured values below detection limit

Table 4. Results (p-values) of single factor ANOVA.

Variable	Collection time	Comparing vegetation types for each location		Comparing across sites for each substrate	
		Site	P	Vegetation	P
% OM remaining	t=6	Upland	<0.001*	Maple	0.027*
		Marsh	0.711	Site-specific	0.002*
		Swamp	0.363		
	t=10	Upland	<0.001*	Maple	0.025*
		Marsh	<0.001*	Site-specific	0.001*
		Swamp	0.197		
	t=16	Upland	0.101	Maple	0.109
		Marsh	0.796	Site-specific	0.046*
		Swamp	0.015*		
C:N	t=6	Upland	<0.001*	Maple	<0.001*
		Marsh	0.002*	Site-specific	<0.001*
		Swamp	0.001*		
	t=10	Upland	0.005*	Maple	<0.001*
		Marsh	0.005*	Site-specific	<0.001*
		Swamp	0.737		
	t=16	Upland	0.149	Maple	<0.001*
		Marsh	0.003*	Site-specific	<0.001*
		Swamp	0.971		
DOC mg g ⁻¹ OM	t=10	Upland	<0.001*	Maple	0.015*
		Marsh	0.190	Site-specific	<0.001*
		Swamp	0.167		

*indicates statistical significance (p<0.05)

Table 5. Results (F ratios and p-values) from single factor ANOVA comparing bacterial respiration across sites at each time period. Mean respiration rates (nmoles CO₂ g⁻¹ OM hr⁻¹) and standard errors are shown for each site, and superscript letters denote statistically significant subgroups as determined by a Tukey's post hoc comparison.

Collection period	F	P	Upland	Marsh	Swamp
t=3	21.19	<0.001*	0.2 ± 0.1 ^a	0.1 ± 0.0 ^a	24.1 ± 5.2 ^b
t=6	13.79	0.002*	0.2 ± 0.0 ^a	7.2 ± 0.4 ^b	9.8 ± 2.7 ^b
t=10	21.62	<0.001*	0.3 ± 0.1 ^a	5.7 ± 0.8 ^b	3.5 ± 0.6 ^b
t=16	17.56	<0.001*	1.3 ± 0.2 ^a	4.3 ± 0.4 ^b	2.4 ± 0.5 ^a

*indicates statistical significance (p<0.05)

Table 6. Results (F ratios and p-values) from single factor ANOVA comparing fungal respiration across sites at each time period. Mean respiration rates (nmoles CO₂ g⁻¹ OM hr⁻¹) and standard errors are shown for each site, and superscript letters denote statistically significant subgroup as determined by a Tukey's post hoc comparison.

Collection period	F	P	Upland	Marsh	Swamp
t=3	36.80	<0.001*	0.3 ± 0.1 ^a	0.1 ± 0.0 ^a	9.4 ± 1.5 ^b
t=6	16.02	0.001*	0.3 ± 0.1 ^a	2.5 ± 0.1 ^a	12.2 ± 2.7 ^b
t=10	10.21	0.005*	0.7 ± 0.1 ^a	3.1 ± 0.2 ^b	2.3 ± 0.6 ^b
t=16	11.51	0.003*	0.8 ± 0.1 ^a	1.6 ± 0.7 ^a	3.2 ± 0.1 ^b

*indicates statistical significance (p<0.05)

Table 7. Results (p-values) of two-tailed t-tests comparing bacterial and fungal respiration at each site over time.

Collection period	Upland	Marsh	Swamp
t=3	0.900	0.242	0.035*
t=6	0.008*	<0.001*	0.524
t=10	0.026*	0.044*	0.050*
t=16	0.050*	0.001*	0.287

*indicates statistical significance (p<0.05)

Table 8. Results (r-value) from Pearson correlation analysis of the upland site.

	% OM remaining	DOC	Bacterial respiration	Fungal respiration	Bacteria axis 1	Bacteria axis 2	Fungi axis 1	Fungi axis 2	C:N
% OM remaining		0.253	-0.775*	-0.846*	-0.562	-0.201	0.039	-0.436	0.125
DOC			-0.483	-0.398	0.368	-0.493	-0.494	-0.648	0.637*
Bacterial respiration				0.7100*	0.887*	-0.357	-0.497	0.615	-0.311
Fungal respiration					0.462	-0.025	-0.195	0.171	-0.582
Bacteria axis 1						-0.297	-0.582	0.614	-0.169
Bacteria axis 2							0.334	0.083	-0.240
Fungi axis 1								-0.295	-0.375
Fungi axis 2									-0.499

*indicates significant (<0.005)

Table 9. Results (r-value) from Pearson correlation analysis of the marsh site.

	% OM remaining	DOC	Bacterial respiration	Fungal respiration	Bacteria axis 1	Bacteria axis 2	Fungi axis 1	Fungi axis 2	C:N
% OM remaining		0.481*	-0.178	-0.316	0.089	-0.597	0.294	0.167	0.589*
DOC			-0.680	-0.766*	0.679	-0.492	0.211	-0.355	0.845*
Bacterial respiration				0.861*	-0.532	-0.035	-0.192	0.695	-0.103
Fungal respiration					-0.691	-0.057	-0.060	0.274	-0.413
Bacteria axis 1						0.607*	-0.355	-0.367	-0.046
Bacteria axis 2							-0.569	-0.253	-0.678*
Fungi axis 1								-0.276	0.278
Fungi axis 2									0.154

*indicates significant (<0.005)

Table 10. Results (r-value) from Pearson correlation analysis of the swamp site.

	% OM remaining	DOC	Bacterial respiration	Fungal respiration	Bacteria axis 1	Bacteria axis 2	Fungi axis 1	Fungi axis 2	C:N
% OM remaining		0.273	0.426	-0.093	-0.542	0.237	-0.370	-0.207	0.358
DOC			0.669	0.263	-0.480	0.073	-0.458	-0.423	0.580*
Bacterial respiration				0.282	-0.538	0.173	-0.582	-0.381	0.742*
Fungal respiration					0.376	0.418	0.087	-0.049	0.145
Bacteria axis 1						0.285	0.798*	0.376	-0.673*
Bacteria axis 2							0.284	0.052	0.185
Fungi axis 1								0.100	-0.581
Fungi axis 2									-0.187

*indicates significant (<0.005)

APPENDIX B: Figures

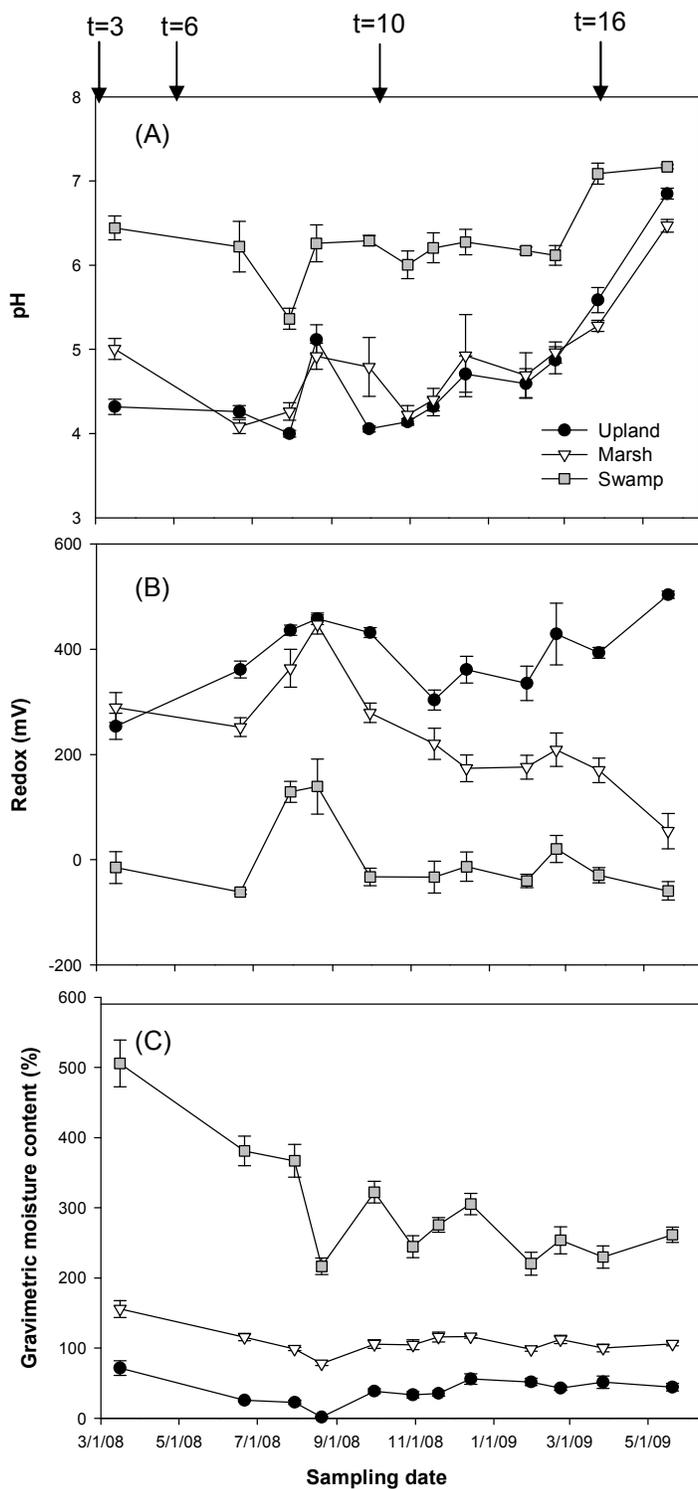


Figure 1. Soil characteristics of each site from monthly sampling: (A) pH, (B) redox potential, and (C) gravimetric moisture content (mean \pm SE).

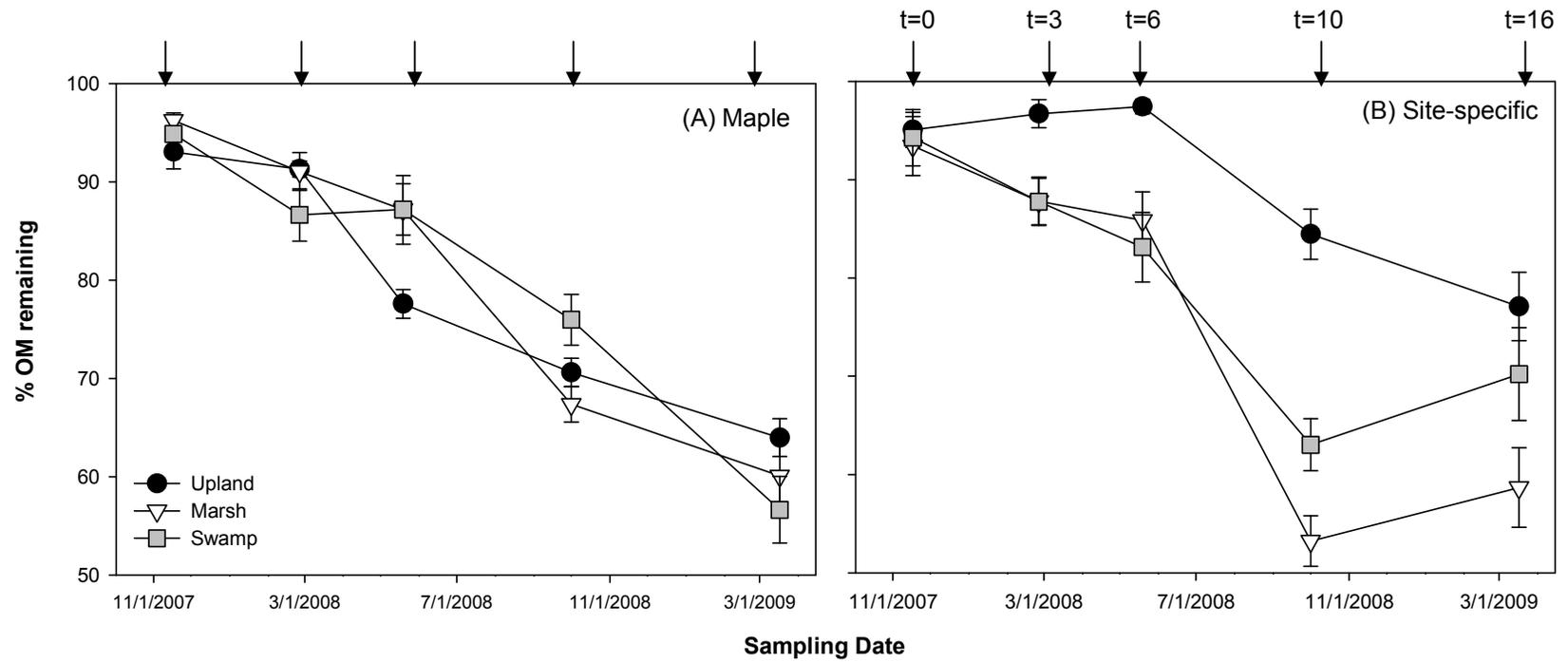


Figure 2. Comparison of percent OM remaining by site over the sampling period: (A) maple leaves and (B) site-specific vegetation (mean \pm SE).

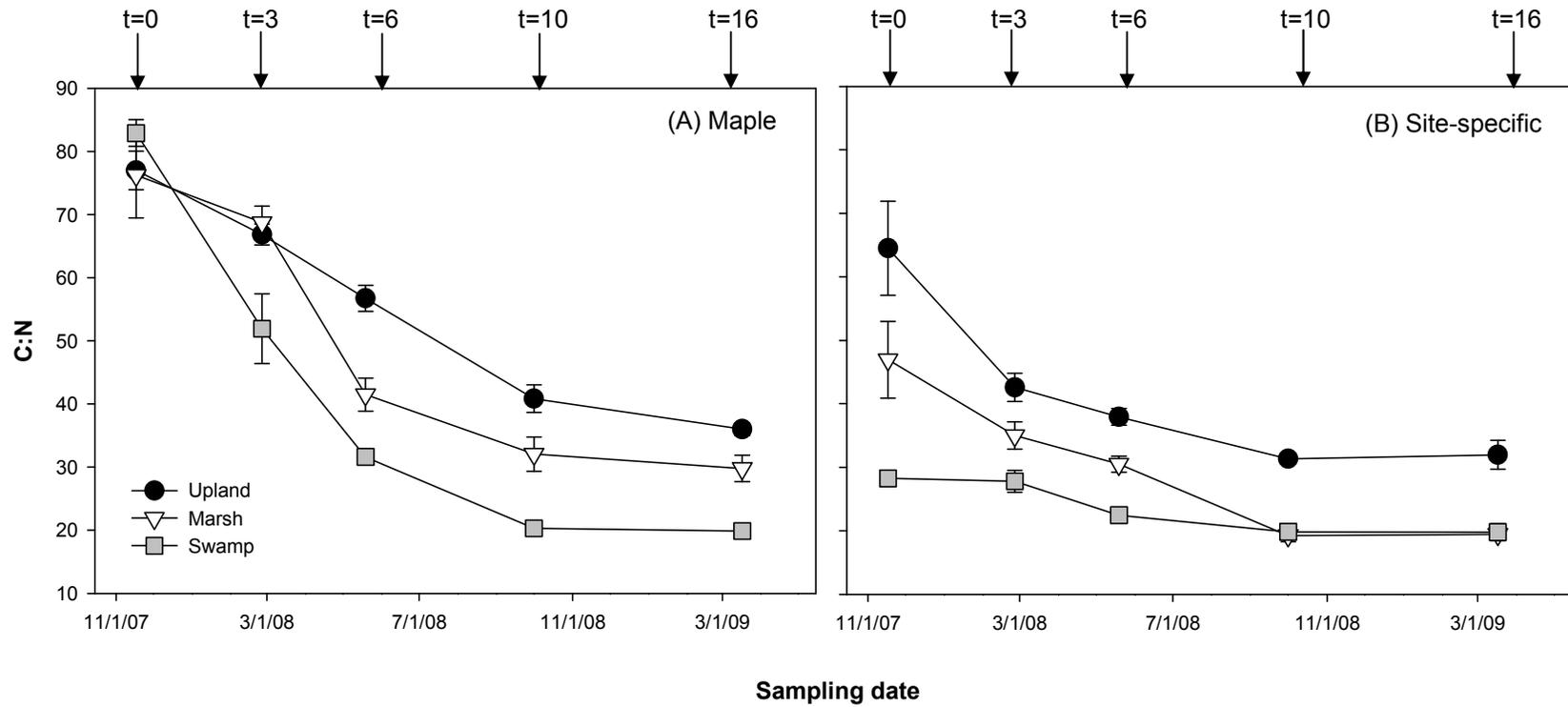


Figure 3. Comparison of C:N by site over the sampling period: (A) maple leaves and (B) site-specific vegetation (mean \pm SE).

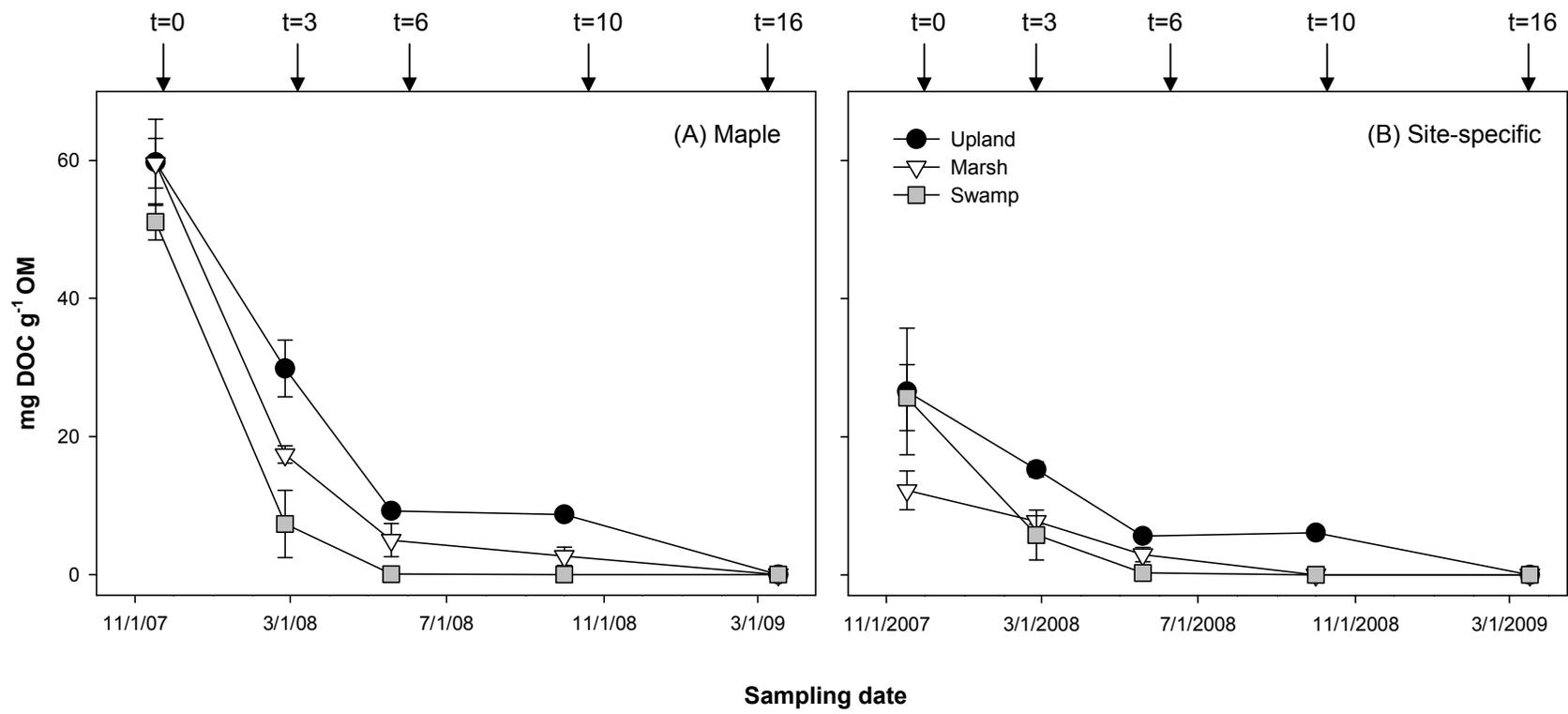


Figure 4. Comparison of DOC released by site over the sampling period: (A) maple leaves and (B) site-specific vegetation (mean \pm SE).

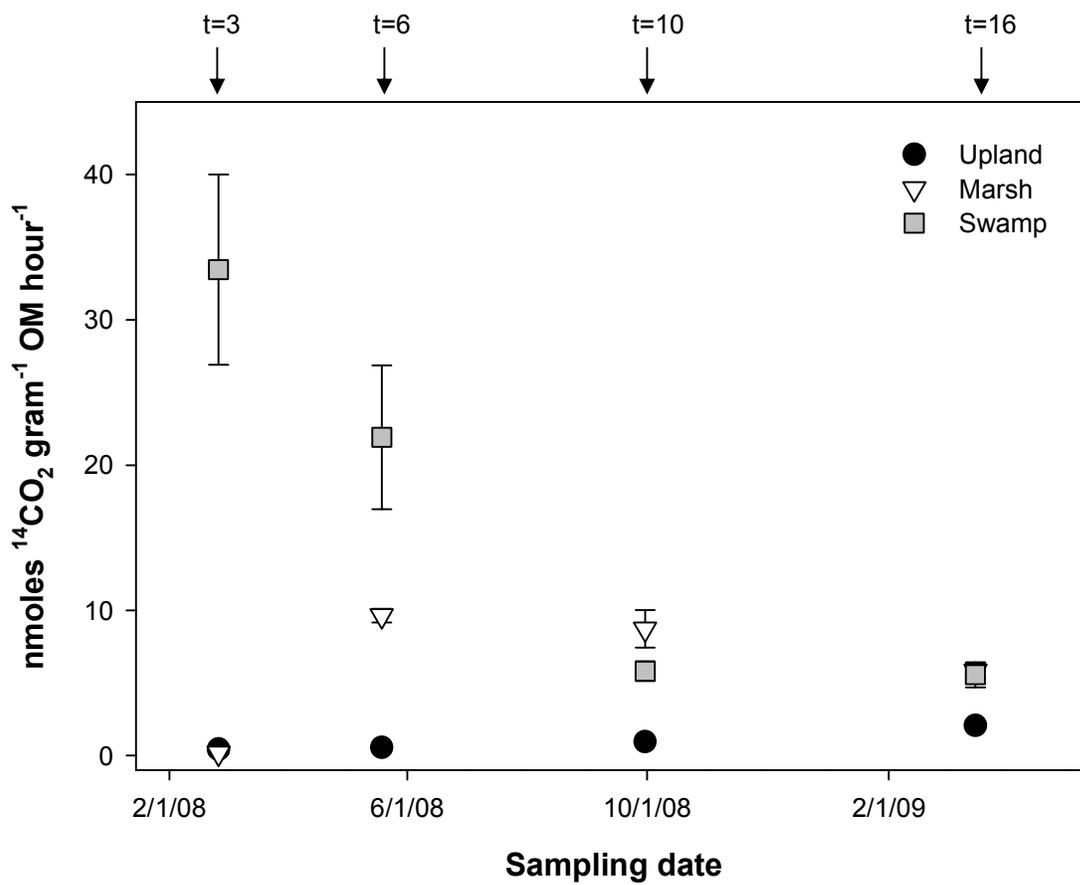


Figure 5. Comparison of the total microbial respiration at each site over the sampling period (mean \pm SE).

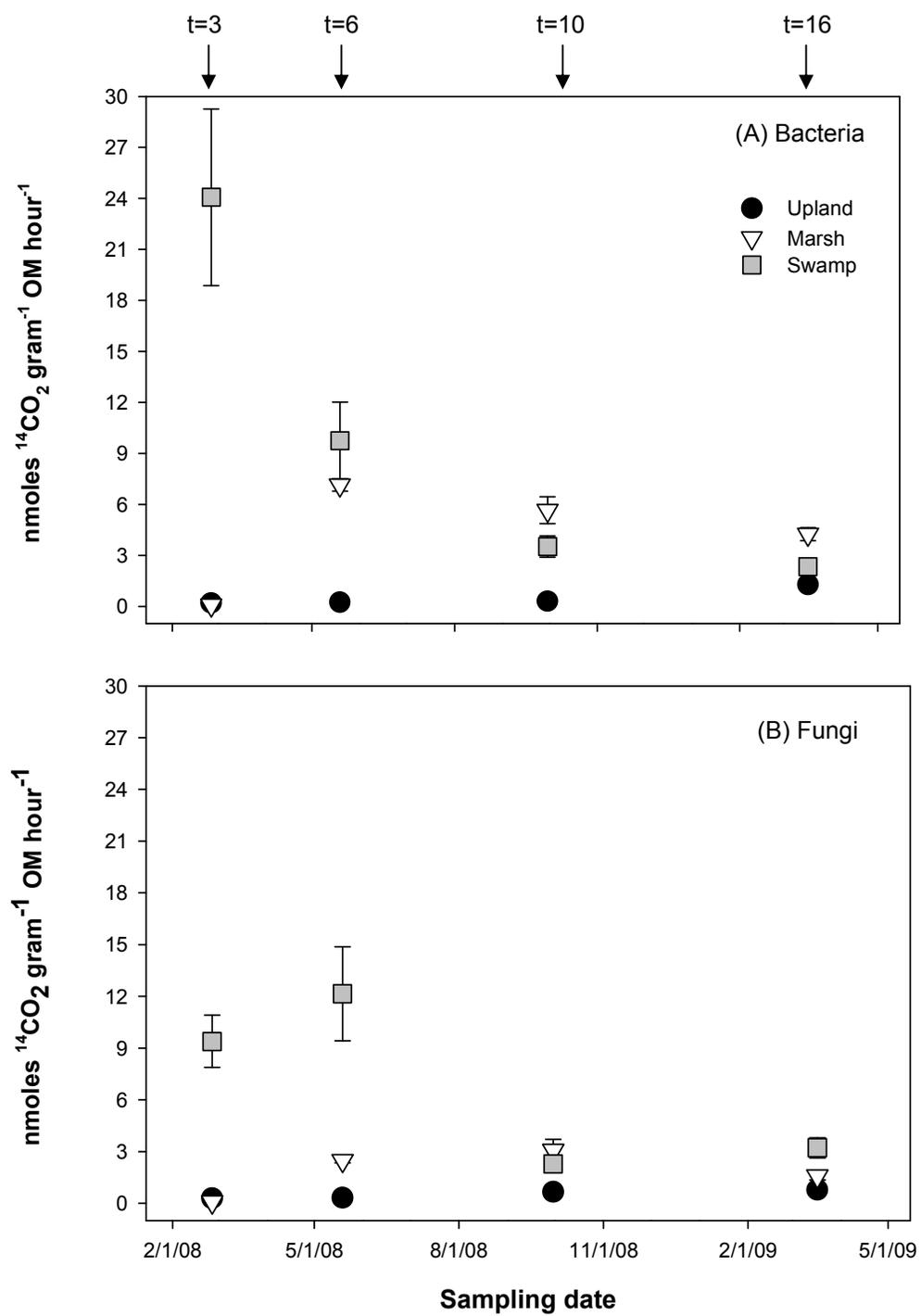


Figure 6. Comparison of respiration rates at each site over sampling period: (A) bacterial respiration and (B) fungal respiration (mean \pm SE).

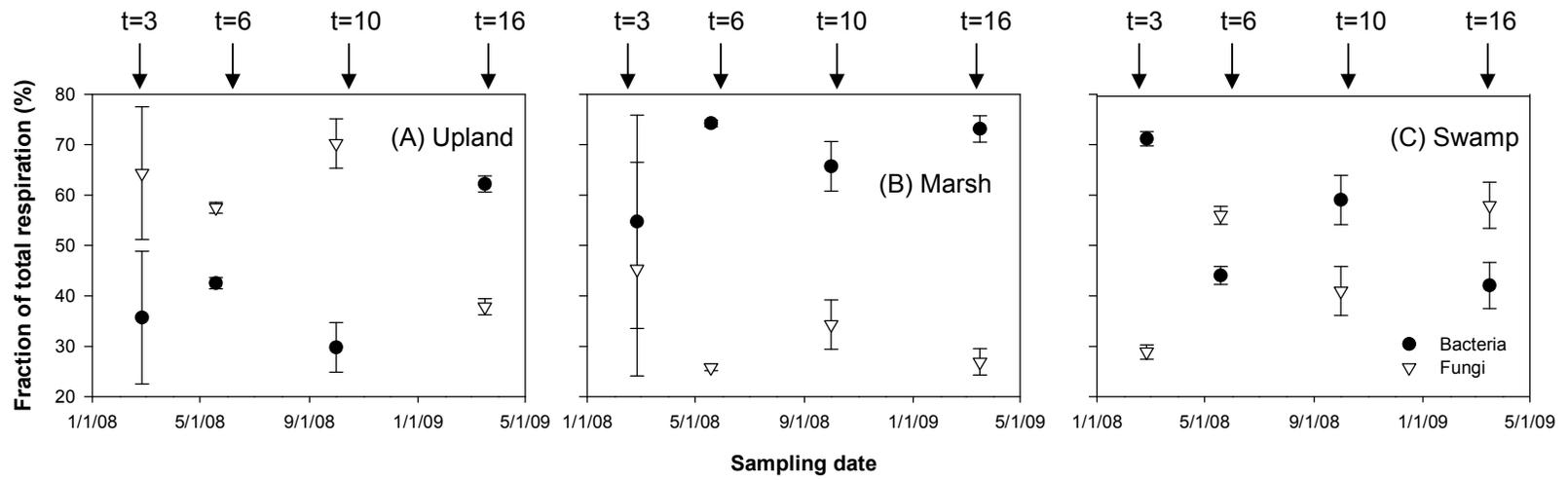


Figure 7. Fraction of total respiration (%) by microbial group for each site: (A) upland, (B) marsh, and (C) swamp (mean \pm SE).

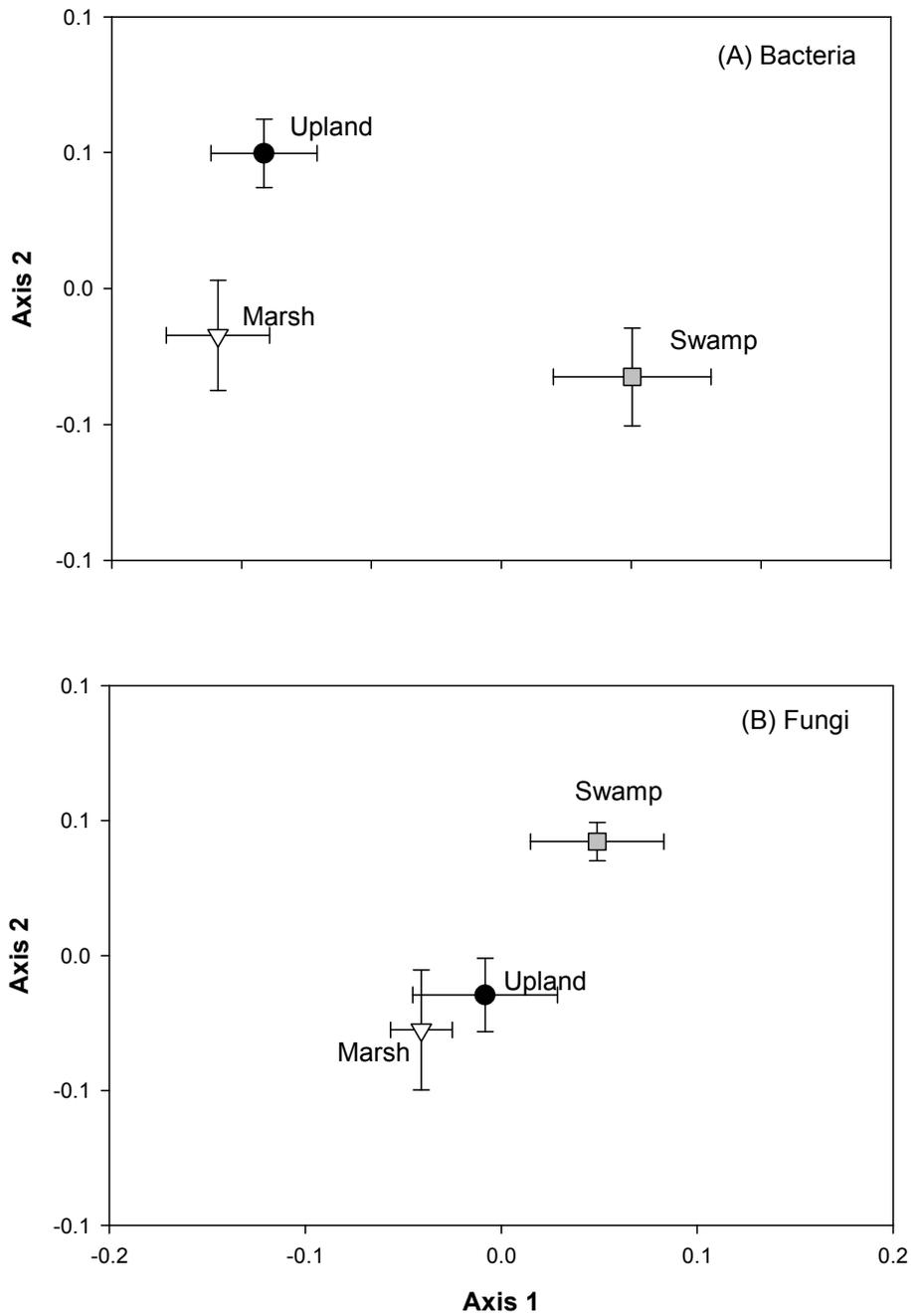


Figure 8. Multidimensional scaling analysis of T-RFLP results. Data for all times grouped by site: (A) bacteria and (B) fungi (mean \pm SE).

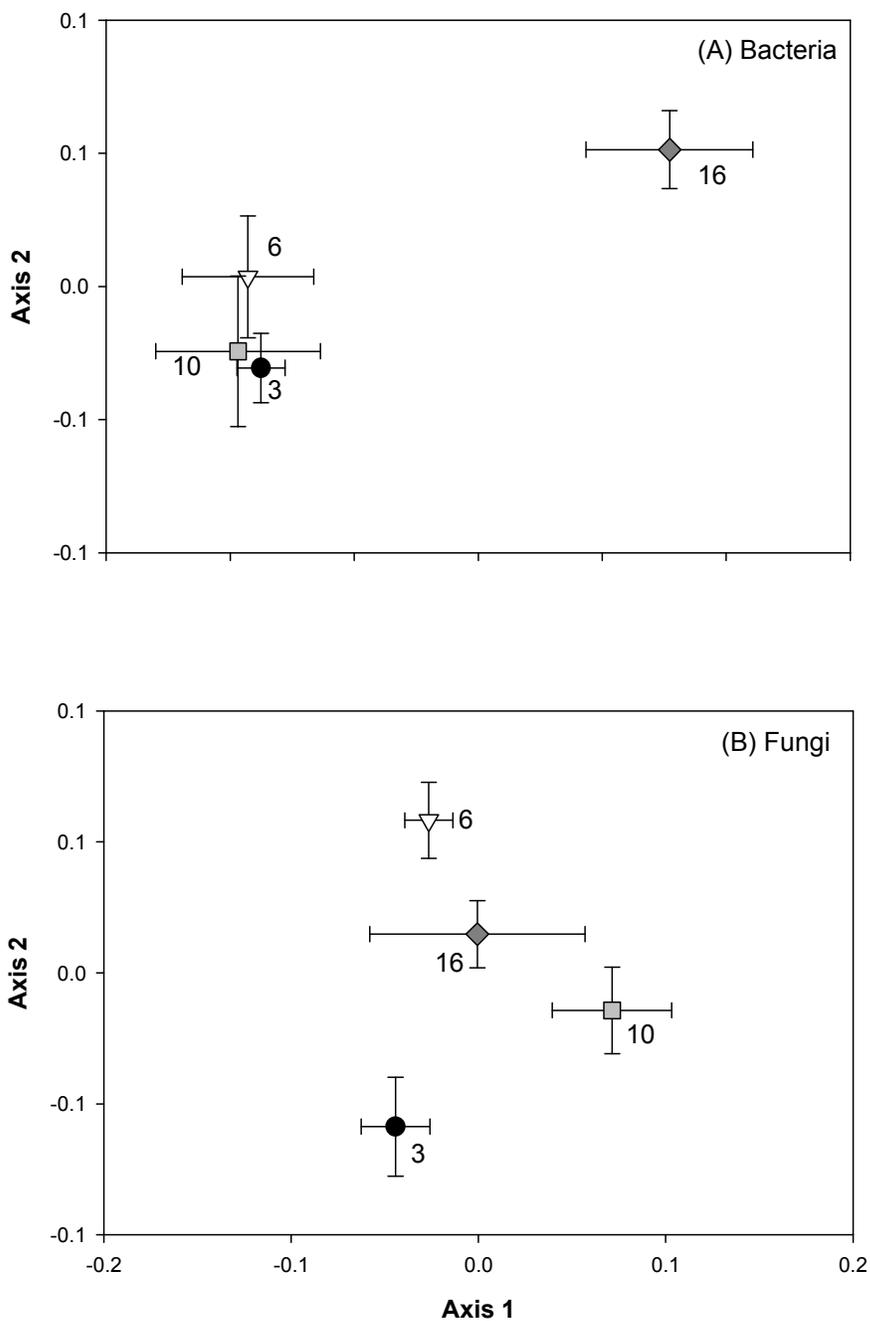


Figure 9. Multidimensional scaling analysis of T-RFLP results. Data for all sites grouped by sampling time: (A) bacteria and (B) fungi (mean \pm SE).

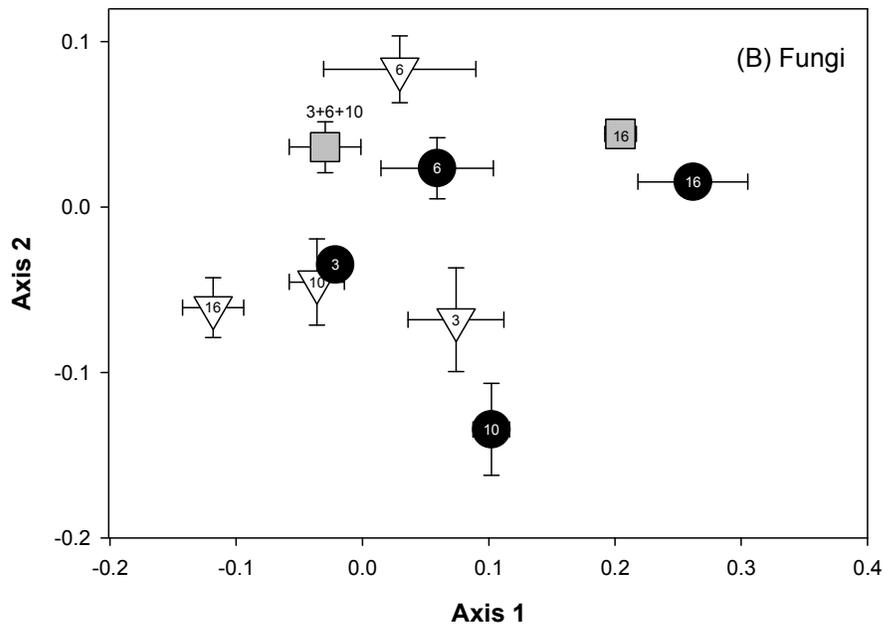
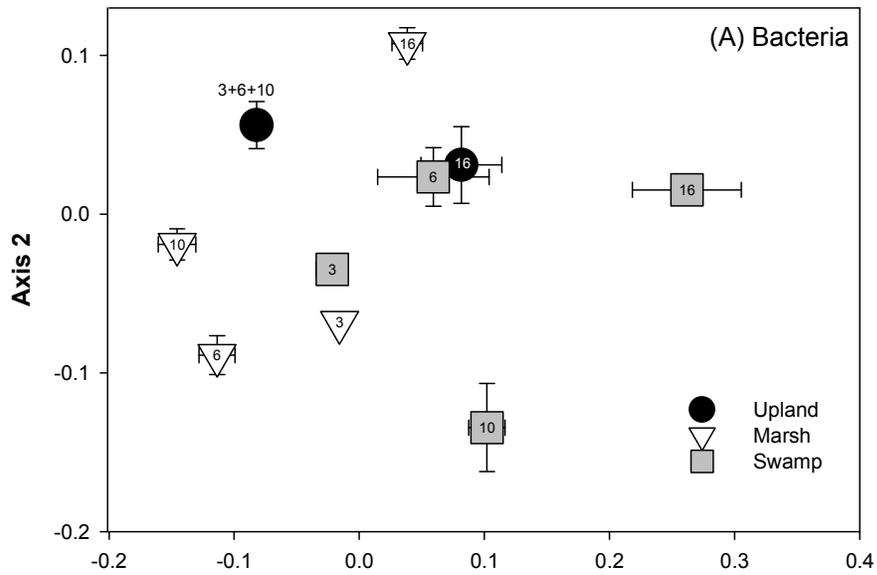


Figure 10. Multidimensional scaling analysis of T-RFLP results. Data grouped by site and collection period: (A) bacteria and (B) fungi. Numbers represent collection period (mean \pm SE).

APPENDIX C: Site locations

GPS coordinates of Sites

Site	Sub-site ID	N°	W°
Upland	A	37.32933	77.20601
Upland	B	37.32919	77.20605
Marsh	C	37.33665	77.20585
Marsh	D	37.33659	77.20596
Swamp	E	37.32844	77.20906
swamp	F	37.32830	77.20900

VITA

Morgan Alice Rawls was born in Woodstock, GA on September 17, 1982. She attended Etowah High School in Woodstock and graduated in 2001. In 2005, she graduated *Magnum cum laude* from Georgia Southern University with a BS in Biology. While attending Georgia Southern, she conducted an undergraduate research entitled “Unraveling of a Plant Species Complex: *Ophiocolea floribunda*”. In 2009, she received her MS in Biology from Virginia Commonwealth University. While at VCU, she made the Dean’s list every semester, received two Rice Center grants, received a Graduate School Thesis/Dissertation Assistantship, and maintained a 4.0 GPA.