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Journal:	<i>Genome</i>
Manuscript ID	gen-2015-0199.R5
Manuscript Type:	Article
Date Submitted by the Author:	03-May-2017
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Is the invited manuscript for consideration in a Special Issue? :	This submission is not invited
Keyword:	microbial ecology, wetlands ecology, eDNA analysis, next generation sequence taxonomy

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Using metagenomics to show the efficacy of forest restoration in the New Jersey Pine Barrens.

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ABSTRACT

The Franklin Parker Preserve within the New Jersey Pine Barrens contains 5,000 acres of wetlands habitat, including old-growth Atlantic White Cedar (or AWC; *Chamaecyparis thyoides*) swamps, cranberry bogs, and former cranberry bogs undergoing restoration into AWC forests. This study showed that the C-use efficiency was greater in the old-growth AWC soils than in soils from 8-year old mid-stage restored AWC stands, which were greater than found in soil from 4-year old AWC stands—the latter two stands being restored from long-term cranberry bogs. A metagenomic analysis of eDNA extracted from these soils showed that the C-cycle trends were associated with increases in the relative numbers of DNA sequences from several copiotrophic bacterial groups (Bacteroidetes and Proteobacteria), complex C decomposing fungal groups (Sordiomycetes, Mortierellales, and Thelephorales), and collembolan and formicid invertebrates. All groups are indicators of successional more advanced soils, and critical for soil C-cycle activities. These data suggest that the restoration activities studied are enhancing critical guilds of soil biota, and increasing C-use efficiency in the soils of restored habitats, and that the use of metagenomic analysis of soil eDNA can be used in the development of assessment models for soil recovery of wetlands following restoration.

Key Words: wetland soils ecosystem restoration, soil community diversity, carbon use

INTRODUCTION

Scientists around the world are developing and attempting to use indicator methods to characterize ecological differences between terrestrial habitats, to assess ecosystem condition within these habitats, and to identify the impact ecosystem restoration and other land management strategies may have on them. The advent of next-generation sequencing (NGS) platforms, such as Illumina MiSeq, make it feasible to conduct more detailed analyses of the biotic composition of environmental soil or water samples than could have occurred previously. This has made it possible to address important questions that link the effects of environmental disturbances with the role of the soil biota in nutrient cycling in different ecosystems (Hajibabaei 2012; Baird and Hajibabaei 2012; Gibson et al. 2014; Hajibabaei et al. 2011, 2012; Shokralla et al. 2012). As a result of these advances in the ability to study biotic communities, it is possible to examine the details of how biotic change influences the nutrient cycles as habitats shift from intact to disturbed to restored conditions, and how such biotic metrics, in concert with more traditional C cycle metrics, will be able to show the efficacy of remediation strategies on disturbed lands.

Wetland ecosystems are greatly disturbed lands across the planet, yet, are highly productive systems, accumulate large below-ground stocks of carbon (C), occupy 7% of total land area on the planet, contribute 10% of the planet's net primary productivity, and have a great potential for exchange of greenhouse gases with the atmosphere (Strom et al. 2005; US Climate Tech Prog 2003). Increasing concern over global climate change has generated interest in identifying existing and prospective C sinks (Euliss et al. 2006), such as wetlands, but little is known of the ecology of wetlands soils as C sinks, despite the recognized importance of wetland soils on global C budgets (Mitsch and Gosselink 2007). The State of New Jersey lost about 60%

of its wetlands since the 1870's (Balzano et al 2002; US Fish and Wildlife 2005), and still continues to lose more, as New Jersey's remaining palustrine wetlands and estuarine wetlands (US Fish and Wildlife 2005) are experiencing increasing stress. The New Jersey Department of Environmental Protection (NJDEP) established a strategic planning goal to improve wetland quality and function through wetlands creation, enhancement, restoration, preservation, and banking (Balanzo et al. 2002).

Chamaecyparis thyoides (Atlantic white-cedar, or AWC) is a dominant and important tree species within the wetlands of the eastern United States and originally covered 200,000 ha there (Kuser and Zimmermann 1995; Rodgers et al. 2004), including about 46,000 ha in New Jersey (New Jersey Forest Service 1997). The AWC is an obligate wetland plant (Reed 1988), whose swamp forests are characterized by seasonal flooding and a hummocked microtopography that includes both aerobic and anaerobic microhabitats, with saturated soils and/or small creeks or streams in between the hummocks (Cantelmo and Ehrenfeld 1999). The health and stability of the AWC communities are sensitive to both excessive flooding and drought (Cantelmo and Ehrenfeld 1999). The AWC serves as the keystone species in these swamps, influencing understory light levels, microtopography, climate, and the composition of other plant and animal species populations (Rodgers et al. 2004). Unfortunately, the populations of AWC in the eastern United States have declined by as much as 95% in some areas since the European settlement (Gengarelly and Lee 2005; Rodgers et al. 2004). Heavy cutting of AWC for lumber, shingles, and shipbuilding occurred in the New Jersey Pine Barrens (NJPB) as early as the seventeenth century (Harshberger 1971; Laderman 1989; Forman 1998); and many of the former AWC swamps were then converted into cranberry bogs in the 1800s (Forman 1998).

The decline of AWC in New Jersey has resulted in recent interest in its management and restoration (Gengarelly and Lee 2005; New Jersey Forest Service 1997; Mylecraine et al. 2004; Mylecraine et al. 2005). The Franklin Parker Preserve within the NJPB was acquired by the New Jersey Conservation Foundation (NJCF) in 2004. In the fall of 2004, the NJCF began the physical restoration of 1,100 acres of former AWC swamp habitats that had been converted into cranberry bogs and blueberry fields, after which AWC root cuttings were planted beginning in the summer of 2005.

Studies on the efficacy of these restoration activities have not previously been conducted. Such studies should include an assessment of the below-ground communities, as it is known that when land management practices decrease vegetation diversity and structural complexity, they also decrease the soil ecosystem condition by causing a qualitative and quantitative loss of soil C, which can be associated with a shift in soil biotic diversity, biomass, and community structural complexity, and affect the partitioning of above- and below-ground C (Wardle 2006; Kardol and Wardle 2010; Girvan et al. 2003,). Recently, scientists have identified that although there are important relationships that occur between soil biota and the trees and plants in wetlands worldwide, which play fundamental roles in establishing and sustaining these wetland plant communities, these relationships have been little studied and are poorly understood (Ipsilantis and Sylvia 2007; Wang et al. 2010; Wang et al. 2011).

The soil biotic community is known to play important roles in nutrient cycling, which influences plant community composition (Wardle 2006; Kardol and Wardle 2010; Wardle et al. 2004; Fierer et al. 2007,). The soil fungi decompose complex organic substrates more efficiently than bacteria, leaving behind recalcitrant residues and enhancing the organic carbon matter in the soil, and are more important as decomposers in older or restored soils (Sinsabaugh 2010; Talbot

et al 2008). The soil bacteria are more critical in decomposition and N nutrient cycling in managed, young, or recovering ecosystems (Moore and de Ruiter 1991; Lovell et al. 1995). The soil invertebrate community greatly affects critical functions of the soil microbial communities, such as recycling of organic matter, decomposition, and primary production, thereby increasing the efficiency of C utilization (Lavelle, 1996; Brussaard, 1998; Cragg and Bardgett, 2001; Chamberlain et al., 2006; Wang et al., 2009). In particular, soil fauna accelerates microbial access to C and non-C nutrients through exposure to fecal material rich in substrates and other microbes (McGee and Eaton, 2014; Chapin et al., 2011; Lavelle et al., 1997; Wang et al., 2009), and through a variety of other processes (Brussaard, 1998; McGee and Eaton, 2015; Chapin et al., 2011; Wang et al., 2009). How the activities of these three important soil communities will respond to environmental change and restoration efficacy is a critical research question for predicting future ecosystem function and climate feedbacks.

A study was conducted to assess how the restoration of former cranberry bogs into AWC stands has affected the soil invertebrate, fungal, and bacterial community composition and biomass, and critical indicators of the C cycle activities, within this old growth and restored stands of AWC in the NJCF. Study plots were established in AWC old-growth stands and regions restored in 2004 and 2008 within the NJCF region in the NJPB, where some of the largest areas of naturally occurring AWC cedar swamps occur (Kuser and Zimmerman 1995). It was hypothesized that the old-growth AWC stands would have more complex soil biotic communities and be more efficient at C utilization than the other stands, and that there would be a trajectory towards these conditions from the young to the intermediate restored AWC stands. A simple DNA extraction method was used along with NGS of the DNA, combined with analysis of traditional C component metrics, to compare the ecological conditions of the different habitats

and observe patterns of enhanced efficiency of C utilization, in association with detailed soil biotic community structure analyses along the land management gradient.

METHODS

PLOT DESIGN AND HABITATS STUDIED

The habitats for this study were divided into plots using the standard stratified, block, systematic plot study design used in forestry, environmental assessment of damaged lands, and restoration ecology, as recommended by the American Society of Foresters (ASF; see www.forestandrange.org) and the US Environmental Protection Agency (see EPA 2002 document Guidance on choosing a sampling design for environmental data collection--2002, EPA/240/R-02/005). These, and other sources, state that stratified sampling of stands is appropriate for studies of damaged lands, natural experiments (i.e., effects of flooding or tornados), and land manipulations such as restoration efforts. ASF defines a stand as "*A distinct, recognizable management unit of a forest consisting of a contiguous area that is relatively uniform in species composition, age, structure, treatment, etc.*". The mean data resulting in inferential statistical analyses collected from plots within the "stand type" can be compared to that found within other and different "stand types".

We followed the general guidelines of ASF with respect to stand identification and size and number of plots per stand. Thus, each plot represents a true replicate with that stand of habitat, for that larger region only. As stated in Hurlburt (1984) and by Davies and Gray (2015), the results of such comparative mensurative studies can be used for characterizing and comparing smaller-scale habitats of ecological interest; otherwise, how would one compare a marsh, unusual forest patch, natural disturbance such as a gap due to wind, remediation

efforts, all within a single forest type? In these situations, there is only 1 large stand type to be compared to the undisturbed forest. As recommended by these and other authors and published research studies, the only way possible to study these unusual and ecologically important sites is to consider that unique habitat as a stand, divide it into appropriate size plots (based on ASF recommendations), and consider each plot as a replicate to represent the conditions within that unique stand. The Editorial Board of Ecology and Evolution, after description of a specific study on red pines, stated that *"If the population for this experiment were defined as 'this red pine stand', then the block design would indeed have 4 true replicates."* Thus, in keeping with these definitions, we have used the phrase within "this stand of Atlantic White Cedar...".

This original 2004 AWC planted area that we used was considered the mid-stage restored site for the current study (39°46'33.28"N, 74°32'58.22"W). The 2008 AWC planted area was used as the young restored sites used for the current study (39°46'32.19"N, 74°31'44.26"W). These restoration areas are adjacent to intact old-growth AWC swamps (39°46'13.90"N, 74°32'8.33"W) which were used in this study. The three plot types were selected based on their potential reproductive activity as suggested by Mylecraine et al. (2004). Specifically, the young restored AWC would be pre-reproductive, the mid-stage restored AWC would be early reproductive, and old-growth AWC stands would be fully reproductive.

In the summer of 2011, four 400 m² plots were established in each of the above-described AWC stands—young restored, intermediate restored, and old-growth AWC. Using a standardized grid format, the plots were divided into twenty-five 4 m x 4 m sections, and a 2 cm (diameter) x 15 cm (depth) soil core was collected from the central point of each section. The soil samples were pooled by plot. After collection, the large bag of soil from a plot was emptied onto a sterile surface, and thoroughly mixed to provide an even distribution of soil samples

collected from each of the 4 plots per habitat type. The soil was sieved through 4 mm mesh at field moist levels, and mixed again, then used for the various analyses. The rate of soil respiration (as mg CO₂/g dry soil/hr) was determined using the closed system titration methods of Alef and Nannapieri (1995) and 10 g of dry weight equivalent soil samples. The soil biomass C (μg C/g), was determined by the standard fumigation-extraction methods of Jenkinson (1988) as the difference between K₂SO₄ extracted organic C levels in ethanol-free chloroform-fumigated and unfumigated 10 g of dry weight equivalent soil samples. The organic C levels (as μg C/g) were determined from 10 g of dry weight equivalent soil samples by the Walkley-Black rapid dichromate procedure, modified by Nelson and Sommers (1996). From these, the microbial metabolic quotient ratios as qCO₂ (ratio of CO₂ from respiration/C_{mic}) were determined to estimate the efficiency of utilization of organic C. The lower the qCO₂, the more efficient the microbial community is using organic C, and is presumably converting more C into biomass, rather than releasing it as CO₂ (Anderson 2003). The CO₂ flux was determined as the μmol CO₂/m²/sec using a LiCor 8100 CO₂ flux analysis system.

ENVIRONMENTAL DNA SEQUENCING ANALYSIS

Environmental DNA (eDNA) was extracted from three 0.3g replicate field moist soil samples from each of the raw homogenized soil samples per habitat type, using the MoBio PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) and eluted with 70 ul of elution buffer. The concentration and purity (A₂₆₀/A₂₈₀ ratio) of extracted soil eDNA were determined using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA). Soil eDNA was sequenced using the Illumina MiSeq (Illumina Inc., San Diego, California, USA) platform, targeting the 16S ribosomal RNA gene region for bacteria and archaea

(Caporaso et al. 2011), the nuclear internal transcribed spacer (ITS) ribosomal RNA gene region for fungi (Gardes and Bruns 1993), and the standard 5' end of the mitochondrial protein-coding gene cytochrome *c* oxidase subunit I (COI) for arthropods (Hajibabaei et al. 2012). The 16S gene regions v3F and v4R were amplified by Polymerase Chain Reaction (PCR) using one primer set targeting two non-overlapping variable gene regions v3 (~197 bp) and v4 (~288 bp) using the primer set [16S v3F, 5'-ACTCCTACGGGAGCAGCAG-3'; 16S v4R, 5'-GGACTACARGGTATCTAA T-3' (Sundquist et al. 2007)]. The ITS gene region was amplified by PCR targeting the full ITS regions using the primer set [ITS4, 5'-TCCTCCGCTTATTGATATGC-3'; ITS1F 5'-CTTGGTCATTTAGAGGAAGTAA-3' (White et al. 1990)]. One sequencing direction of the ITS amplicons was used to target the ITS1 region, and the other direction was used to target the ITS2 region. The 658 bp standard 5' end of COI was amplified targeting three fragments: F230 (~230 bp), BR5 (~330 bp), and BR (~450 bp), using three primer sets {[F, 5'-GGTCAACAAATCATAAAGATATTGG-3' (Folmer et al. 1994); 230, (CTTATRTRTTTATTCGIGGAAIGC (Gibson et al. 2014), [B, 5'-CCIGAYATRGCITTYCCICG-3' (Hajibabaei et al. 2012); R5, 5'-CCIGTIYTIGCIGGI GCIATYAC (Gibson et al. 2014)], [B, 5'-CCIGAYATRGCITTYCCICG-3' (Hajibabaei et al. 2012); R, 5'-TAAACTTCAGGGTGACCAAAAATCA-3' (Folmer et al. 1994)]}.

Amplicons were prepared using a two-step PCR process. The first step was performed with the target-specific primers. Each PCR amplification contained 2 µL DNA template, 17.5 µL molecular biology grade water, 2.5 µL 10x reaction buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4), 1 µL 50x MgCl₂ 50 mM), 0.5 µL dNTPs mix (10 mM), 0.5 µL forward primer (10 mM), 0.5 µL reverse primer (10 mM), and 0.5 µL Invitrogen Platinum Taq polymerase (5 U/µL) in a total volume of 25 µL. The PCR conditions for 16S and ITS were 95 °C for 5 min; 35 cycles of

94 °C for 40 s, 46 °C for 1 min, and 72 °C for 30 s; and 72 °C for 5 min; and held at 4°C. The PCR conditions for COI were initiated with heated lid at 95°C for 5 min; followed by a total of 30 cycles of 94°C for 40s, 46°C (for all 3 primer sets) for 1 min, and 72°C for 30s; and a final extension at 72°C for 5 min, and hold at 4°C. PCR products were visualized on 1.5% agarose gels to confirm successful amplification by the presence of fluorescent bands under a UV spectrophotometer. PCR products were then purified using a Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA, USA) and eluted in 30 µL of molecular biology grade water. A second PCR step was implemented using the purified 1st PCR product as a template and with Illumina adaptor-tailed, target-specific primers. The 2nd PCR was made following the same protocol as aforementioned except for 30 cycles were used for PCR. All PCRs were done using Eppendorf Mastercycler ep gradient S thermal cyclers, and negative DNA extraction (no sample) and negative PCR control reactions (no DNA template) were included in all experiments. All generated soil amplicons plates were dual indexed and sequenced in several Illumina Miseq runs using a V2 Miseq sequencing kit (500 cycles - 250 × 2)(FC-131-1002 and MS-102-2003). Generated sequence data were submitted to DRYAD (deposited March 1, 2017; doi:doi:10.5061/dryad.mt048).

The 16S Illumina-generated sequences were paire-ended according to the size of the amplicons using SEQPREP software (<https://github.com/jstjohn/SeqPrep>) requiring a minimum overlap of 25bp and no mismatches, and quality filtered and primers trimmed using PRINSEQ v0.20.4 (Schmieder & Edwards 2011) with a minimum of 20 Phred score (99% base call accuracy) with a window of 10, and a step of 5. The passed quality-filtered sequences were denoized, to remove the PCR and sequencing random errors, and clustered for potential delineation at the individual species level with USEARCH (Edgar 2010) at 99% similarity to

establish the OTUs to be used, with de novo chimeric sequences removed. Subsequently, the 99% similarity 16S OTU clusters were taxonomically identified using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) with the RDP project default bootstrap cutoff value of at least 95% similarity. Illumina-generated forward and reverse fragments of ITS sequences were analyzed separately. Forward and reverse ITS sequences were quality filtered and primers trimmed using PRINSEQ v0.20.4 (Schmieder and Edwards 2011) with a minimum of 10 Phred score with a window of 10, and a step of 5. The ITS Phred scores are generally lower than other genes as the amplicons have a greater tendency to form loops when they are single-stranded in NGS. The passed quality-filtered sequences were denoized, and then clustered with USEARCH (Edgar 2010) at 99% similarity level to establish the OTUs to be used, and de novo chimeric sequences removed. Subsequently, the 99% ITS OTU clusters were taxonomically identified using the MEGA-BLAST algorithm (Zhang et al. 2000). These taxonomic identifications of the 99% ITS OTU clusters were assigned based on a minimum percent identity of 90%, against a reference library of ITS sequences in GenBank, word size of 28, and a minimum *E*-value of $1e-20$. For each COI sample, the forward and reverse raw reads for the F230, BR, and BR5 fragments were merged with SEQPREP software (<https://github.com/jstjohn/SeqPrep>) requiring a minimum overlap of 25bp and no mismatches. Illumina-generated COI sequences were quality filtered and primers trimmed using PRINSEQ v0.20.4 (Schmieder and Edwards 2011) with a minimum of 20 Phred score with a window of 10, and a step of 5. Likewise, the passed quality-filtered sequences were denoized and clustered with USEARCH (Edgar 2010) at 99% similarity, and de novo chimeric sequences removed. The F230, BR, and BR5 clusters were pooled, and to identify OTUs, the centroids for each cluster were identified using the MEGA-BLAST algorithm (Zhang et al. 2010), at 99% similarity against a reference library of all COI sequences

downloaded from the GenBank Database (downloaded on Jan 29 2015) with a minimum length of 100bp. All generated sequences were not rarefied as this has been shown to bias sequencing results and data (McMurdie and Holmes 2014). The identifications were performed to different taxonomic levels for each group, based on both what is most commonly used in the literature or the taxonomic level to which important functions could be applied. For bacteria, this was at the phylum level, as it is one of the most commonly used for this group. For fungi, when possible, the taxa were identified to the family level. However, it is commonly recognized that fungal taxonomy is experiencing changes. Consequently, there are times when the information within the databases identify fungal genera from the DNA sequence, but not the family, and sometimes not the class, as these taxonomic classifications are still being debated. In these situations, we identified the taxa as an unknown family within a specific order or class. The invertebrate sequences were generally identified to order, but for those specific sequences whose relative abundance levels differed by >10% between habitats, the taxonomic level examined was genus.

STATISTICAL ANALYSES

The mean values for each C cycle metric were determined from the 4 plots per forest type, and the significance of the differences were analyzed by ANOVA and Tukey's HSD *post-hoc* test using SPSS software (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.). The relative percent occurrence of each unique DNA sequence-based taxonomic OTU (as the % sequence hits) from the different taxonomic groups was calculated per soil sample. From these data, the similarities in the biotic community composition across the three habitat types were evaluated by analysis of variance (ANOVA) and Standardized Effect Size (SES) using the software EcoSim (v.7; Gotelli and Entsminger, 2005). Square root

transformed DNA sequence-based taxonomic OTU relative percent occurrence data were used in a similarity percentage analysis (SIMPER) in Primer E (v.6, Plymouth, UK) to demonstrate the percent that each taxonomic group was contributing to the differences between habitats. The Primer-E BEST (Bio-Env + Stepwise) function was used to determine if the highest rank correlations (ρ) found between the biotic similarity matrix and the matching distance matrix from the C cycle variables occurred at levels that exceed that which would be expected to occur by chance under the null hypothesis of no real biotic-environment link.

RESULTS

The data show very clear differences in the C cycle metrics measured (Table 1, Figure 1), with a trend toward increased efficiency in C utilization across the restoration gradient, within this old growth and restored stands of AWC in the NJCF. The soil organic C and soil biomass C were significantly greater, and the $q\text{CO}_2$ and CO_2 flux significantly lower in the soils from the old-growth AWC habitats than the other two AWC stands. The significantly lowest levels of soil organic C and soil biomass C, and significantly greatest levels of $q\text{CO}_2$ and CO_2 flux, were found in the soils from the young restored AWC stands. The mid-stage restored AWC soils had values of soil organic C, soil biomass C, and $q\text{CO}_2$ intermediate between, but significantly different from, the other two AWC stands.

The sequencing of the soil DNA resulted in a mean of 59,992 reads (range of 34,631 – 88,715) for bacterial 16s sequences (mean size of 399 bp; range of 328 - 419 bp); a mean of 74,920 reads (range of 40,566 – 96,862) for fungal ITS sequences (mean size of 239 bp; range of 221 – 252 bp); and a mean of 222,746 reads (range of 187,696 – 248,882) for invertebrate COI

sequences (mean size of 248 bp; range of 208 – 257 bp). The results of the ANOVA and SES analyses of the bacterial, fungal, and invertebrate DNA sequences showed that there were statistically significant differences in the biotic community composition between the 3 habitats studied (Table 2). The analysis of the bacterial DNA analysis from the three different habitats resulted in identification of 21 phyla from the samples (Table 3). The DNA sequences from Acidobacteria, Bacteroidetes, and Proteobacteria were the most represented in the samples, with about 5-50% sequence hits. The SIMPER analysis demonstrated that DNA sequences from Bacteroidetes, Proteobacteria, and Cyanobacteria contributed the most to the differences in composition between the old-growth and mid-stage and mid-stage and young AWC stand soils, while sequences from Nitrospirae, Bacteroidetes, and Proteobacteria were the greatest contributors to the differences in composition between the old-growth and the young AWC stand soils (Table 4). The results of the NGS analysis of the fungal DNA from the three different habitats resulted in identification of DNA sequences from 4 phyla and 14 families of fungi from the samples. Clear differences in this community were observed from these data (Table 5). There was a gradient of increasing representation of DNA sequences from Ascomycota moving from the young restored AWC to the old-growth AWC soils. Greater abundances of DNA sequences from Basidiomycota were found in the mid-stage AWC, while the amounts of this phylum were relatively the same for the other two habitats. The DNA sequences from Glomeromycota (which are composed of all arbuscular mycorrhizal fungi) were in far greater abundance in the young stand AWC soils than either of the other sites. The DNA sequences from the Mortierellaceae, families in the Sordiomycetes, and families in the Thelophorales, all of which are important complex organic C decomposers, were in greater abundance in the old-growth, followed by the mid-stage, and then the young restored AWC stand soils. The fungal community in the old-

growth and the young restored stand soils were the most separated, followed by the old-growth and mid-stage stands and then the mid-stage and the young restored stands. The SIMPER analysis (Table 6) demonstrated that DNA sequences from the Mortierellaceae, Magnaporthaceae, and unknown different families from the Sordiomycetes and Thelophorales contributed the most to the differences in fungal community composition between the old-growth and mid-stage AWC stand soils. The DNA sequences of unknown families from the order Archaeosporales/phylum Glomeromycota, from the class unknown Sordiomycetes, and representatives from the Mortierellaceae contributed the most to the dissimilarity in composition between the old-growth and the young AWC stand soils. The DNA sequences of representatives from the Glomeromycota, Helotiales, Thelophorales, and Sordiomycetes contributed the most to the differences in fungal composition between the mid-stage and the young restored AWC stands. If one combines the sequence abundances of the Agaricales, Sebinaceae, Tricholomataceae, Theolophorales, and Dothideomycetes, from Table 5, as these are known to contain many ectomycorrhizal (ECM) fungi, then the old-growth AWC stands contained a percent relative abundance (RA) of 42.59% of potential ECM fungi; the mid-stage restored AWC contained a percent RA of 46.28% of these groups; and the young restored AWC contained a percent RA of 38.41% of these groups. Similarly, if one uses Table 5 and combines the sequence abundances of the Mortierellaceae, Magnaporthaceae, other Sordiomycetes, Thelophorales, Tricholomataceae, Agricales, and Helotiales, from Table 5, as these are known to contain many wood-decomposing fungi, then the old-growth AWC stands have an 86.33% RA of DNA sequences from these groups; the mid-stage AWC stands have a 66.50% RA of them; and the young restored stands have a 50.31% RA of the potential woody decomposer groups.

The results of the NGS analysis of the soil invertebrate DNA from the three different habitats resulted in identification of clear differences between the different habitats, especially within the Annelida, Arachnida, Entognatha, and Amphipoda (Table 7). There was a trend of increasing abundance of DNA sequence reads from the various groups of Annelida worms moving from the old-growth to mid-stage restored to young restored AWC stand soils, which had the greatest abundance of DNA sequences from these organisms (collectively, 30.8% RA, 61.8% RA, and 76.6% RA, respectively). It is of particular interest to note that the presence of sequence reads of the enchytraeid species *Cognettia* were found present in greatly increasing levels going from the old-growth AWC to the mid-stage AWC to the young AWC stands (Table 7). Collectively, sequence reads from *Cognettia* sp., the Lumbricidae worm *Aporrectodea* sp., and the Megascolecidae worm *Achaeta* sp. were at the greatest level of abundance in the (Table 7) young restored stands (76.6% RA), followed by the mid-stage (61.8% RA), and then the old-growth AWC stands (30.8% RA). There were also a far greater abundance of reads of the Isotomidae collembolans (37.7%, 1.30%, 0.60%, respectively) and Formicidae members of the Hymenoptera of the genus *Myrmecina* (11.9%, 0.4, 0.3% RA, respectively) in the old-growth AWC stand soils than in the soil from the other two sites. The SIMPER analysis (Table 8) demonstrated that the differences in the abundance of the different worms, collembolans, and hymenopterans contributed the most to the differences in the invertebrate community composition between the old-growth and mid-stage and old-growth and young restored AWC stand soils (Table 4).

The BEST analysis (Table 9) showed that, when combined, the measurement of the soil organic C, qCO₂, and Biomass C were fairly good predictors of the soil biotic community composition, while, interestingly, CO₂ flux was not. These three C cycle metrics were only

modestly correlated with the bacterial community composition ($\rho = 0.219$, $p = 0.11$). They were, however, much stronger predictors of both the fungal and invertebrate community structure ($\rho = 0.777$ and 0.486 ; $p = 0.01$ and 0.03 , respectively).

DISCUSSION

Healthy wetlands exhibit high levels of productivity, accumulate large below-ground stocks of C, and serve as C sinks rather than C sources, thus providing a significant potential source for enhancing primary productivity and C sequestration (Strom et al., 2005; US Climate Tech Prog 2003). Despite this, little is known of the below-ground ecology and C cycle dynamics occurring in these fragile systems (US Climate Technology Program 2003; Strom et al. 2005; Mitsch and Gosselink 2007). For many decades, vast amounts of these critical habitats have been poorly managed and/or destroyed (Balzano et al 2002; US EPA 2008; Mitsch and Gosselink 2007; US Fish and Wildlife 2005). Fortunately, restoring lost wetlands and protecting remaining wetlands appears to be becoming a priority in the US (U.S. Climate Technology Program, 2003; US EPA 2008). However, to fully understand how such wetlands management has affected the C cycle dynamics within these habitats, one must gain knowledge about the biota that are most associated with the mechanisms of this cycle and how they are impacted by different wetlands management practices. Wardle (2006) and Kardol and Wardle (2010) recently recognized the need to identify the linkages between above and below-ground diversity to understand nutrient resource availability, specific habitat properties, and ecosystem condition. These types of studies are needed to demonstrate relationships associated with the biota and C source utilization, as it has significant implications for habitats serving as C sources or sinks. The use of NGS for diversity and community ecology studies has provided easily accessible biotic

information about forest soils that has previously not been logistically reasonable to acquire, allowing for studies that can begin to address the complicated relationships between the C cycle, the soil biota associated with it, and the effects that different land management strategies may have on these soils (Hajibabaei 2012; Baird and Hajibabaei 2012; Hajibabaei et al. 2011; Hajibabaei et al 2012; Shokralla et al. 2012; Gibson et al. 2014;).

The projects occurring in the NJCFs Franklin Parker Preserve within the NJPB represent important efforts to protect existing and restore severely damaged AWC wetland habitats. This current study represents the first project, to our knowledge, to demonstrate how the restoration of a wetlands, previously disturbed for commercial purposes, effects below-ground biota and the C cycle dynamics using 3 different trophic groups, bacterial, fungal, and invertebrate, within a single study and set of soil samples collected at the same time. The overarching goal of this work was to determine if there were possible biotic and abiotic indicators of soil ecosystem recuperation in these systems. The data from this project provide a very clear indication that the metrics employed can serve as indicators that restoration activities implemented in the cranberry bogs within the Franklin Parker Reserve are having a positive effect on the recuperation of the ability of the soils to more efficiently use organic C, which is associated with changes in the soil community composition towards biota that are closely linked to more efficient use of C, and the habitats as C sinks. The next step in this work is to examine the DNA sequences at deeper taxonomic levels to determine if specific genera of invertebrates, fungi, and bacteria can be connected to specific C cycle processes in a trophic cascade model. It is hoped that this will provide insight into the ecological drivers of the dynamics of this cycle.

The amount of organic C and C biomass were greatest in the old-growth AWC soils, and the qCO_2 and CO_2 flux lowest in these soils. The opposite was found to be the case in the young

restored AWC soils, with the mid-stage restored AWC generally being intermediate in these metrics. The greater C biomass and organic C levels suggest an increase in the amount of C being made available for transfer up the food web, while the lower $q\text{CO}_2$ and CO_2 flux levels are important indicators that more efficient use of this C is occurring in these soils (Brookes 1995; Anderson 2003; Anderson and Domsch 1989; Moscatelli et al. 2005). Thus, the restoration activities occurring within these habitats are having positive effects on the ability of these soils to serve as C sinks.

Consistent with the C cycle data are the changes in biotic composition within the different soils. The ANOVA and SES analyses showed very distinct differences in the bacterial communities between the 3 habitats. The SIMPER analyses showed that two of the most important contributors to the differences between habitats were in the phyla Bacteroidetes and Proteobacteria. Many representatives from both these groups are copiotrophic and associated with richer organic C levels in soils (Prosser 2012; Fierer et al. 2007; Lennon et al. 2012;). Thus, it is not surprising that the greater amounts of these sequences were found in the old-growth AWC stand soils. Although the distribution of the bacterial groups seemed to be logical and consistent with the C data, the Primer-E BEST analysis did not provide strong evidence for correlations between the composition of this community and C cycle metrics. It has been suggested that often the bacterial functional groups might be defined primarily at family and phyla level, rather than at species or genera (Moorhead and Sinsabaugh, 2006; Follows and Dutkiewicz 2011; Fierer et al. 2007; Philippot et al. 2010). Thus, it may be that for more specific identification of connections between C metrics and bacterial biota, it may be necessary to “drill down” to the family or genus level, as these level of taxa may be where C cycle functions could be associated with the biotic groups. This is currently being examined for this data set.

The trend towards increasing vegetation diversity being associated with enhancement of C use efficiency was seen along the AWC restoration gradient (young restored to old-growth AWC), as indicated by the organic C, biomass C, qCO_2 , and CO_2 flux data. These results were supported by the DNA sequence analyses as this C-use efficiency should result in development of more recalcitrant compounds, which would select for fungi that degrade these materials (Sinsabaugh 2010; de Boer et al. 2005; Bradford et al. 2008). This was found to be the case as there were greater levels of known complex woody organic C user fungi found along the restoration gradient towards the old-growth AWC stands (50.31% to 86.33%). Members of the Sordiomycetes, Thelophorales, Mortierellaceae, Tricholomataceae, Agaricales, and Helotiales, all groups known to include many complex C decomposers, were increasingly distributed along the restoration gradient, provided important contributions to the differences in composition between habitats along the restoration gradient, and were correlated with the C data from the young restored to the old-growth AWC stand soils (BEST analysis). In addition, the strikingly greater levels of Glomeromycota within the young restored AWC soils were consistent with these habitats containing vastly greater amounts of herbaceous vegetation in comparison to the other habitats. The Primer-E BEST analysis showed very strong and significant correlations between the organic C, biomass C, and qCO_2 levels and the fungal community composition. All these data suggest that examining the fungal community composition at the family level, using NGS methodologies, in concert with C-use efficiency metrics can serve as good indicators of soil ecosystem condition within these soils.

There was a trend in the data from the current study towards increasing abundance of collembolan and hymenopteran (Formicidae) invertebrates, and a decreasing level of enchytraeid worms, moving from the young restored to the old-growth AWC stand soils, and a high level of

importance that these groups played in the contribution of the differences in biota between these three communities (i.e., SIMPER analysis). The ANOVA and SES analyses also showed distinct differences in these soil invertebrate communities between the three habitats studied. The collembolans are important for fragmenting leaf litter and decaying debris in woodlands, and members of the genus *Myrmecina* (hymenopterans), whose DNA sequences were found in this study, are specifically important in rotting wood debris. These soil invertebrates are critical for enhancing the decomposition of complex organic material, making organic C more available to the fungal and bacterial communities and establishing symbiotic relationships with fungi and bacteria (Mueller et al. 2001; Russell et al. 2009). Thus, their increasing level of sequence read abundance found moving from the young restored to the old-growth AWC suggests that they are also playing those same roles in these habitats and could be good biomarkers of restoration. The species of annelids, whose DNA sequence reads were found in this current study, are known to be soil ecosystem engineers and are most common in acidic meadows, peat regions, and disturbed soils (Laasko and Setälä 1991; Augustsson et al. 1998; Briones et al. 1998; Cole et al. 2000; Cole et al. 2002; Liiri et al. 2002), similar to those found in the young restored AWC habitats of the NJPB.

Soil invertebrates are known to play critical roles in the recycling of organic C and on the rates of decomposition in soils by increasing microbial community access to nutrients and enhancing both primary production directly and C use efficiency (Brussaard 1998; Cragg and Bardgett 2001; ; McGee and Eaton 2015; Chamberlain et al. 2006; Lavelle et al. 1996; Lavelle et al. 1997; Wang et al. 2009; Chapin et al. 2011). In particular, members of the meso- and macrofauna play very important roles in this recycling of C (Lavelle et al. 2006). For example,

the mesofauna collembolans enhance the microbial community and C-use efficiency via the fragmentation and ingestion of litter and production of nutrient and microbe-rich fecal material (Lavelle et al. 1997). The macrofauna hymenopterans, such as the ants (Formicidae), physically modify the soil through various mechanisms, enhancing the space and access to nutrients for the microbial community, therefore increasing substrate availability for other faunal and microbial populations (Wardle 2002; Lavelle et al. 1997; Coleman et al. 2004). These two groups, collectively, serve important roles as litter transformers, soil ecosystem engineers, detritivores, and microbivores, resulting in enhanced access to nutrients for the bacteria and fungi, and enhanced C use efficiency. The Primer-E BEST analysis supported this idea in that the composition of the invertebrate community could be directly correlated to the levels of organic C, biomass C, and qCO_2 .

The results of this study show that the restoration activities occurring within the NJPB are enhancing the C-use efficiency within the soils of the restored areas. Furthermore, the data suggest that the soils in the restored regions are changing with a trend towards becoming similar to those in the older AWC forests, and these changes are correlated with certain C-cycle metrics and certain groups of soil biota. At this point, there is a need for more extensive sampling, especially over time and the different seasons of the year, in order to confirm that the C-use efficiency in the restored areas is, in fact, on a trajectory towards similarity with the old-growth AWC forests in the region, and what type of trajectory rate would be expected to reach old-growth AWC forest stand levels. Based strictly on the results from this study, it is difficult to hypothesize a specific time period that will be required for habitat recuperation in the Pine Barrens, but it certainly appears as if the soil C-use efficiency is increasing with time, as are certain groups of soil biota that are likely critical to this increase in efficiency. Given the

differences in C-cycle and biotic metrics between the younger and older restored stands of AWC, we would expect to see a continuing increase in C-use efficiency occurring over space and time.

The “before-during-after” experimental type of design of the restored areas in the NJPB provide an excellent opportunity to measure various components of C-cycle processes and C-use efficiency and to investigate how the interaction between the various soil bacterial, fungal, and invertebrate groups serve as important drivers of these processes. It is very well accepted that soil bacterial populations are early successional biota, involved in early stages of decomposition and N-cycle activities, and are often relatively static in abundance and biodiversity over time (Moore and de Ruiter 1991; Lovell et al. 1995), in comparison to soil fungal and invertebrate populations. The soil fungi are more important as decomposers in older or restored soils (Sinsabaugh 2010; Talbot et al 2008) and interact with the soil invertebrate communities, which increase fungal access to organic C in soils over time (McGee and Eaton, 2014; Lavelle et al. 1997; Wang et al. 2009; Chapin et al. 2011). Given this accepted trophic cascade relationship, especially between the invertebrates and fungi in soil C-use efficiency, and the results of our BEST and SIMPER analyses, we will continue to monitor the qCO_2 , soil biomass, and/or CO_2 flux and look for indicators of increasing C-use efficiency. We will also continue to monitor for an increase in the abundance of different groups of soil invertebrate litter transformers, ecosystem engineers, detritivores, and microbivores, along with an increase in the abundance of complex organic C-decomposing fungi and perhaps ectomycorrhizal fungi, as determined by NGS methodologies.

There is no doubt that damage to the wetlands habitats across the planet has been significant and that protection and restoration strategies need to be employed. In addition, it is important to provide methods for the rapid and accurate assessment of the efficacy of the different restoration strategies. The advent of NGS technology is an important tool that should be used by ecologists and land managers and applied to the understanding of ecological processes and their biotic drivers. This would allow for the enhanced understanding of the effects that damage to various habitats has had around the world and the efficacy of restoration attempts within these habitats.

When land management practices decrease vegetation diversity and structural complexity, they also decrease the soil ecosystem condition by causing a qualitative and quantitative loss of soil C, which can be associated with a shift in soil biotic diversity, biomass, and community structural complexity and affect the partitioning of above- and below-ground C (Wardle 2006; Kardol and Wardle 2010; Girvan et al., 2003,). No models are available that show trends in soil ecosystem improvement following restoration of wetlands. However, in this current study, it has been shown that the use of standard C cycle metrics in combination with NGS-based community composition of the bacterial, fungal, and invertebrate communities, analyzed using ANOVA, SIMPER, SES, and the Primer-E BEST statistics, can provide a suite of methods for the analysis of wetland soil habitat conditions and begin to provide a model for studying soil recovery of wetlands following restoration implementation.

ACKNOWLEDGEMENTS

We especially wish to thank Dr. Emile DeVito and the New Jersey Conservation Foundation for their dedication to restoration of AWC swamps in the Franklin Parker Preserve and for inviting

us to conduct this study. Funding for this project was provided by the Kean University Office of Research and Sponsored Programs, and the Pace University, Dyson School of Arts and Sciences. We would like to thank Dr. Daniela Shebitz (Kean University) and the Kean University undergraduate students Kate Niemiera and Daniel Rockefeller, who helped in the field.

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C Metric	F Statistic	<i>p</i> Value	df
Organic C	29.354	0.0001	3
Biomass C	38.294	0.0002	3
CO ₂ Flux	5.730	0.025	3
qCO ₂	51.753	0.0008	3

ANOVA Post Hoc Tests				<i>p</i> Value
Organic C	Tukey HSD	Old Growth	Mid-Stage	0.00824
			Young	0.00008
		Mid-Stage	Old Growth	0.00824
			Young	0.01245
		Young	Old Growth	0.00008
			Mid-Stage	0.01245
Biomass C	Tukey HSD	Old Growth	Mid-Stage	0.00147
			Young	0.00003
		Mid-Stage	Old Growth	0.00147
			Young	0.01676
		Young	Old Growth	0.00003
			Mid-Stage	0.01676
CO ₂ Flux	Tukey HSD	Old Growth	Mid-Stage	0.27869
			Young	0.01991
		Mid-Stage	Old Growth	0.27869
			Young	0.24223
		Young	Old Growth	0.01991
			Mid-Stage	0.24223
qCO ₂	Tukey HSD	Old Growth	Mid-Stage	0.00970
			Young	0.00001
		Mid-Stage	Old Growth	0.00970
			Young	0.00041
		Young	Old Growth	0.00001
			Mid-Stage	0.00041

Table 1. Results of ANOVA and Tukey HSD *post-hoc* analyses of the difference in the mean values of organic C, Biomass C, CO₂ flux, and qCO₂ values from soils collected from Old Growth Atlantic White Cedar (Old Growth), and Young Restored (Young) and Mid-Stage Restored (Mid-Stage) Atlantic White Cedar stands in the New Jersey Pine Barrens.

2a.

ANOVA and SES Results for Bacterial Diversity				
Habitat Comparison	df	F Stat	<i>p</i> value	SES
Old Growth vs. Young	3	4.35	0.015	6.13
Old Growth vs. Mid-Stage	3	6.52	0.011	8.12
Mid-Stage vs. Young	3	4.96	0.017	6.52

2b.

ANOVA and SES Results for Fungal Diversity				
Habitat Comparison	df	F Stat	<i>p</i> value	SES
Old Growth vs. Young	3	8.43	0.025	8.91
Old Growth vs. Mid-Stage	3	6.81	0.008	8.74
Mid-Stage vs. Young	3	5.02	0.014	6.12

2c.

ANOVA and SES Results for Invertebrate Diversity				
Habitat Comparison	df	F Stat	<i>p</i> value	SES
Old Growth vs. Young	3	5.79	0.001	6.32
Old Growth vs. Mid-Stage	3	6.04	0.016	7.14
Mid-Stage vs. Young	3	5.31	0.028	5.93

Table 2. Results of the ANOVA and SES analysis of the differences in DNA-based bacterial, fungal and invertebrate community composition between the old growth, mid-stage restored and young restored Atlantic White Cedar forest soils. The abundance data were square root transformed and normalized prior to analysis. 2a) is for bacterial 16S-based diversity. 2b) is for fungal ITS-based diversity. 2c) is for invertebrate COI-based diversity.

Phylum	Old Growth	Mid- Stage	Young
Acidobacteria	48.81	52.40	50.63
Actinobacteria	2.06	1.41	1.64
Armatimonadetes	0.06	0.11	0.13
Bacteroidetes	4.40	8.47	5.97
Chlamydiae	0.27	0.57	0.51
Chloroflexi	0.04	0.23	0.04
Cyanobacteria/Chloroplast	0.03	0.92	0.05
Euryarchaeota	0.05	0.17	0.21
Firmicutes	0.15	0.31	0.14
Gemmatimonadetes	0.04	0.00	0.04
Hydrogenedentes	0.00	0.00	0.01
Microgenomates	0.00	0.06	0.03
Nitrospirae	0.00	0.00	0.22
Parcubacteria	0.00	0.06	0.03
Planctomycetes	0.24	0.73	0.61
Proteobacteria	39.82	30.77	36.85
Spirochaetes	0.01	0.00	0.00
Tenericutes	0.01	0.00	0.00
Thaumarchaeota	0.02	0.08	0.07
unclassified	0.00	0.04	0.00
Verrucomicrobia	3.99	3.69	2.83

Table 3. The relative percent of the DNA sequences identified to specific bacterial phyla in sample DNA extracted from soils within old growth, mid-stage restored, and young restored Atlantic White Cedar forest stands in the New Jersey Pine Barrens.

Old Growth & Mid-Stage Average dissimilarity = 11.95%			
Bacterial Phyla	Old Growth Avg. Abund.	Mid-Stage Avg. Abund.	% Contribution of Difference
Bacteroidetes	>		14.86
Cyanobacteria		>	14.31
Proteobacteria	>		13.97
Old Growth & Young: Average dissimilarity = 7.35%			
	Old Growth Avg. Abund.	Young Avg. Abund.	% Contribution of Difference
Nitrospirae		>	14.14
Bacteroidetes	>		10.54
Proteobacteria	>		9.65
Mid-Stage & Young: Average dissimilarity = 8.32%			
	Mid-Stage Avg. Abund.	Young Avg. Abund.	% Contribution of Difference
Cyanobacteria	>		18.81
Proteobacteria	>		13.36
Bacteroidetes	>		11.89

Table 4. The results of SIMPER analysis in Primer-E (ver. 6) software comparing the relative percent (%) contribution of the top three bacterial phyla (as determined by DNA sequence-based taxonomic identification) to the difference in bacterial community composition between the Old Growth, Mid-Stage Restored, and Young Restored Atlantic White Cedar forest soils within the New Jersey Pine Barrens.

Phylum	Old Growth	Mid Stage	Young
Ascomycota	43.80	31.20	21.80
Basidiomycota	46.00	65.20	43.80
Glomeromycota	0.20	0.90	31.40
Zygomycota	9.60	2.20	2.60

Family			
Unknown Dothideomycetes Family	1.87	1.19	3.66
Helotiaceae	2.11	0.48	2.29
Unknown Helotiales Family	10.25	15.55	6.48
Unknown Sordiomycetes Family	12.80	5.40	4.30
Sordiomycetes--Magnaporthaceae	10.96	0.28	0.09
Unknown Ascomycota Family	11.14	5.36	4.33
Clavariaceae	0.06	0.03	0.92
Tricholomataceae	0.31	3.13	1.46
Sebacinaceae	0.00	2.47	0.14
Unknown Agricales Family	0.50	3.40	2.50
Unknown Thelephorales Family	39.91	36.09	30.65
Unknown Basidiomycota Family	3.98	1.96	1.33
Unknown Archaeosporales-Glomeromycota Family	0.18	0.90	31.40
Mortierellaceae	9.49	2.17	2.54

Table 5. The relative percent of the DNA sequences identified to specific fungal phyla and families (by DNA sequence-based taxonomic identification) in sample DNA extracted from soils within old growth, mid-stage restored, and young restored Atlantic White Cedar forest stands in the New Jersey Pine Barrens.

Old Growth & Mid Stage: Average dissimilarity = 25.77%			
Family	Old Growth Avg. Abund.	Mid Stage Avg. Abund.	% Contribution of Difference
Magnaporthaceae (Sordiomycetes)	>		18.9
Mortierellaceae	>		10.91
Unknown Sordiomycetes Family	>		8.51
Unknown Thelophorales Family		>	8.26
Old Growth & Young: Average dissimilarity = 29.40%			
Family	Old Growth Avg. Abund.	Young Avg. Abund.	% Contribution of Difference
Unknown Archaeosporales-Glomeromycota Family		>	29.99
Magnaporthaceae (Sordiomycetes)	>		17.46
Unknown Sordiomycetes Family	>		8.72
Mortierellaceae	>		8.61
Mid Stage & Young: Average dissimilarity = 22.66%			
Family	Mid Stage Avg. Abund.	Young Avg. Abund.	% Contribution of Difference
Unknown Archaeosporales-Glomeromycota Family		>	36.24
Unknown Helotiales Family	>		10.89
Unknown Thelophorales Family	>		9.9
Unknown Sordiomycete sFamily	>		9.32

Table 6. The results of SIMPER analysis in Primer-E (ver. 6) software comparing the relative percent (%) contribution of the top four fungal families (as determined by DNA sequence-based taxonomic identification) to the difference in fungal community composition between the Old Growth, Mid-Stage Restored, and Young Restored Atlantic White Cedar forest soils within the New Jersey Pine Barrens.

Taxa	Old Growth	Mid Stage	Young
<u>Annelida</u>			
Enchytraeidae (>95% <i>Cognettia</i> species)	27.30	47.90	61.20
Lumbricidae (> 95% <i>Aporrectodea</i> species)	1.90	0.30	11.90
Megascolecidae (>92% <i>Achaeta</i> species)	1.60	13.60	3.50
<u>Archnida</u>			
Araneae	0.20	1.60	0.20
Orbatida mites	2.00	1.30	4.60
<u>Entognatha</u>			
Collembola (>92% Isotomidae)	37.70	1.30	0.60
<u>Insecta</u>			
Coleoptera	1.80	3.40	1.90
Diptera	1.60	5.00	0.50
Hymenoptera(Formicidae, >95% <i>Myrmecina</i>)	11.90	0.04	0.03
Lepidoptera	0.70	1.40	1.30
<u>Amphipoda</u>	0.30	0.20	1.50

Table 7. The relative percent of the DNA sequences identified to specific invertebrate taxa (using DNA-sequence-based taxonomic identification) in sample DNA extracted from soils within old growth, mid-stage restored, and young restored Atlantic White Cedar forest stands in the New Jersey Pine Barrens.

Old Growth & Mid-Stage: Average dissimilarity = 36.91%			
Taxa	Old Growth Abund.	Mid-Stage Abund.	% Contribution of Difference
Collembola (springtails)	>		30.84
Hymenoptera(Formicidae)	>		20.05
Megascolecidae		>	14.95
Enchytraeidae		>	10.46
Old Growth & Young: Average dissimilarity = 36.50%			
Taxa	Old Growth Abund.	Young Abund.	% Contribution of Difference
Collembola (springtails)	>		33.07
Hymenoptera(Formicidae)	>		20.2
Enchytraeidae		>	16.02
Lumbricidae		>	12.77
Mid-Stage & Young: Average dissimilarity = 25.51%			
Taxa	Mid-Stage Abund.	Young Abund.	% Contribution of Difference
Lumbricidae		>	27.25
Megascolecidae	>		17.06
Diptera	>		14.36
Orbatida mites		>	9.43

Table 8. The results of SIMPER analysis in Primer-E (ver. 6) software comparing the relative percent (%) contribution of the top four invertebrate groups (as determined by DNA sequence-based taxonomic identification) to the difference in invertebrate community composition between the Old Growth, Mid-Stage Restored, and Young Restored Atlantic White Cedar forest soils within the New Jersey Pine Barrens.

Bacterial Community		
rho	Variables	<i>p</i> value
0.219	organic C, qCO2,biomass C	0.11
Fungal Community		
rho	Variables	<i>p</i> value
0.777	organic C, qCO2,biomass C	0.01
Invertebrate Community		
rho	Variables	<i>p</i> value
0.486	organic C, qCO2,biomass C	0.03

Table 9. The results of the BEST analysis in Primer-E (ver. 6) software to correlate the bacterial, fungal, and invertebrate communities to the carbon cycle variables that best predict the composition of the biotic community in soil samples from the New Jersey Pine Barrens.

1a) **1b)**

1c)

1d)

Figure 1. Carbon metric data from old growth, mid-stage restored, and young restored Atlantic White Cedar (AWC) forest soils within the New Jersey Pine Barrens. 1a) Soil organic carbon levels. 1b) Soil biomass carbon levels. 1c) qCO_2 values. 1d) CO_2 flux levels.

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