ROLE OF DISSOLVED ORGANIC CARBON IN DETERMINING BACTERIAL COMMUNITY STRUCTURE AND FUNCTION IN AQUATIC ECOSYSTEMS: IS STRUCTURE RELATED TO FUNCTION

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DEDICATION

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Heterotrophic bacteria play a key role in uptake and mobilization of dissolved organic carbon (DOC) in aquatic ecosystems (Tranvik, 1990; Findlay, 2010). DOC requirements of bacteria can be met by various autochthonous and allochthonous sources. Primary producers like algae, are sources of autochthonous DOC while external inputs of dissolved or particulate organic matter represent allochthonous carbon sources. Prior studies suggest that bacteria in lakes can utilize autochthonous DOC most efficiently (Kritzberg et al., 2004, 2005) while allochthonous inputs are generally more important in stream ecosystems (Tank et al., 2010). However, autochthonous and allochthonous DOC pools are heterogenous in composition and can differentially effect bacterial community structure and function (Strauss and Lamberti, 2002; Olapade and Leff, 2005; Docherty et al., 2006; Judd et al., 2006). This is because bacterial communities are physiologically diverse and respond differently to the varied organic carbon sources (Lennon and Cottingham, 2008). As a result, detailed investigations are required to understand the effect of heterogeneity in DOC sources on bacterial responses.

In aquatic ecosystems, differences in bacterial response to major DOC sources, i.e., autochthonous and allochthonous inputs, have been generalized based on the type of
ecosystem. For example, while lotic ecosystems, such as streams and rivers, are predicted to be most influenced by terrestrial or allochthonous inputs (Tank et al., 2010; Pollard and Ducklow, 2011), lentic ecosystems are assumed to be dominated by autochthonous primary production (Kritzberg et al., 2004, 2005; Maki et al., 2010). However, allochthonous DOC can be important in lakes (Berggren et al., 2010; Maracelli et al., 2011) and autochthonous production can be important for stream bacteria as well (Risse-Buhl et al., 2011).

Bacterial responses vary seasonally as autochthonous and allochthonous inputs change over time (Lennon and Pffaf, 2005; Besemer et al., 2005). However, other environmental variables, beyond DOC sources, also change seasonally making it difficult to ascertain cause and effect relationships in field surveys. Also, bacterial response to DOC is based on physiological requirements that may depend on environmental factors, such as temperature, dissolved oxygen or nutrient concentrations (Wever et al., 2005; Apple et al., 2006; Kan et al, 2006; Rubin and Leff, 2007). This makes it important to take multiple environmental factors into consideration while determining the effect of DOC inputs on bacterial communities.

Even when a single form of DOC input is considered (autochthonous or allochthnonous), presence or absence of a dominant DOC source can influence bacterial responses, as is observed in the case of algal blooms in lake ecosystems. The major source of DOC in this case is photosynthetic exudates from the bloom forming algae, comprising of a mixture of carbon compounds such as carbohydrates, amino acids,
proteins, organic acids as well as vitamins (Myklestad, 2000). Most of these compounds are labile forms of DOC and this can lead to competition among bacterial communities for their uptake (Mayfield and Inniss, 1978). Thus, bacterial communities may colonize algae to benefit from proximity to this labile carbon source leading to distinct particle-attached and free-living fractions (Passow et al., 2007; Rink et al., 2007). Easier access to photosynthetic exudates may also result in functional differences between the two bacterial community fractions (Crump et al., 1998; Riemann et al., 2000; Luef et al., 2007).

In any case, DOC in the environment has diverse chemistry, no matter what the source is. Irrespective of the heterogeneity within the allochthonous and autochthonous sources, the DOC pool is often divided into broad size classes based on molecular weight (Amon and Benner, 1996; Malmstrom et al., 2005; Weston and Joye, 2005; Boddy et al., 2008) or bioavailability to microorganisms (Leff and Meyer, 1991; Weigner and Tubal, 2010). The definitions of high and low molecular weights are operational (Amon and Benner, 1996), and do not necessarily relate to bioavailability (Leff and Meyer, 1991). On the other hand, consideration of bioavailability only, does not take into account the molecular complexity of the DOC source. Furthermore, DOC that is bioavailable to some bacteria is not so to others (Cottrell and Kirchman, 2000; Olapade and Leff, 2005). Hence, such broad categorization does not provide a clear idea on the effect of DOC heterogeneity on bacterial responses.
In order to account for both bioavailability and molecular complexity of DOC molecules, attempts have also been made to classify DOC as labile or recalcitrant (Søndergaard and Middleboe, 1995; Currey et al., 2010). Labile DOC comprise of less complex compounds that are easily available to bacteria, while recalcitrant compounds have more complex chemistries and are less bioavailable (Søndergaard and Middleboe, 1995). However, even such a classification, is not clearly defined and is subject to differences in bacterial physiology (Cottrell and Kirchman, 2000). For example, although humic matter is considered to be recalcitrant DOC, it may be equally preferred as a carbon source by some bacteria, compared to more labile compounds (Rosenstock, 2005). This maybe because, for these bacteria, the recalcitrant compounds are more reliable sources of carbon, which cannot be easily taken up by others (Biasi et al., 2005).

Such broad classifications are also problematic because they do not account for variation within a category. For example, both carbohydrates and proteins are categorized as labile compounds (Anno et al., 2000) in spite of differences in chemistry. On the other hand, humic matter, which is recalcitrant, has complex chemistries and varied sources (Thurman, 1985). Therefore, in addition to comparison of bacterial responses among widely differing categories, such as labile versus recalcitrant compounds, smaller differences must be taken into consideration as well, given that bacteria can have differential responses based on DOC chemistry.
Molecular heterogeneity of DOC

DOC is comprised of a wide range of compounds and, as described above, has been classified into broad categories based on molecular weight, chemical complexity and bioavailability. Division into labile and recalcitrant categories is a common form of classification (Søndergaard and Middleboe, 1995; Currey et al., 2010). Based on these criteria, algal exudates are considered labile (Cole et al., 1988; Smith et al., 1995; Grossart and Simon, 1998), and leaf leachates are considered, in some ways, less labile and more recalcitrant (McNamara and Leff, 2004). However, considering the diverse chemistry of compounds even within each category, attempts have been made to classify them further.

Classification of DOC from algae exudates, leaf leachate and soil water derivatives has been carried out using techniques such as ion exchange chromatography (Wallace et al., 2008), fluorescence spectroscopy (Chen et al., 2003) and infrared spectroscopy (Xue et al., 2009). Among these, ion exchange chromatography has been widely employed to study DOC chemistry (Imai et al., 1998, 2001; Wallace et al., 2008), due to its relatively simplistic approach towards classification of DOC based on charge differences between major functional groups (Wallace et al., 2008). The spectroscopic approaches categorize DOC based on the presence or absence of aromatic groups such that the compounds are resolved into categories, like polyphenolic-rich and carbohydrate-rich fractions (Chen et al., 2003). In contrast, ion exchange chromatography facilitates separation of humic and non-humic fractions, as well as separation of the non-humic
acidic, basic and neutral groups. Even the neutral compounds can further be separated as hydrophobic and hydrophilic fractions. In any case, such separation of DOC provides an idea about the heterogeneity of these compounds in the environment.

Humic substances represent the high molecular weight, recalcitrant fraction of dissolved organic carbon and comprise of humic acids, fulvic acids and humin, separated based on their solubility in water at different pH (Thurman, 1985). While fulvic acid is soluble in water under both acidic and basic pH, humic acid precipitates out at pH 2. Humin, on the other hand, is not at all soluble in water. The complexity in chemical structure of humic substances only allows for such an operational approach towards its classification (Tan, 2003). Using synchronous scanning spectrophotometric techniques, the compounds can be divided into different classes of chromophores, owing to the presence of aromatic rings with different functional group substitutions (example, carbonyl, hydroxyl, alkoxy; Peuravuori et al., 2002). Although categorization of humic compounds is not as straightforward as that of non-humic compounds, such attempts using spectroscopic techniques are still indicative of the diversity within this DOC category.

The non-humic fraction of DOC is comprised of comparatively labile compounds, which can be separated based on charge differences of functional groups, by ion-exchange chromatography (Wallace et al., 2008). Accordingly, this broad category of compounds can be acidic, basic or neutral. The acidic fraction mainly refers to compounds with negatively charged functional groups, such as carboxylic acids. The
basic fraction on the other hand is made of compounds with functional groups with positive charges, such as amino acids and their polymers. The hydrophilic neutral groups are parts of compounds, such as monomeric and polymeric carbohydrates. These fractions commonly occur in aquatic ecosystems in different proportions (Findlay and Sinsabaugh, 2003) and lead to the heterogeneity of the DOC pool. Another less commonly occurring class of compounds, namely the organic acids, are categorized as hydrophobic neutrals and also contribute to the chemically diversity of DOC, albeit to a lesser extent (Thurman, 1985).

**Bacterial response to DOC**

Source and quality of DOC has been observed to affect bacterial community composition and metabolic capabilities (e.g., Olapade and Leff, 2005; Docherty et al., 2006). For example, in an experiment conducted by Judd et al. (2006), shifts in structure was observed in bacterial communities from a lake and stream when treated with terrestrial DOC, such that the communities became more similar to those from the source. Also, in that study, terrestrial DOC enhanced lake and stream bacterial production while addition of stream DOC to lake bacteria suppressed production. Another study by Farjalla et al. (2009) demonstrated that freshly leached labile DOC from aquatic macrophytes, led to higher bacterial production, respiration and growth efficiency compared to treatment with humic DOC sources. Furthermore, treatments with the mixture of the two DOC sources led to higher production and respiration rates compared to single treatments.
Such differences in bacterial response to DOC may have been reflective of substrate quality and the source.

Bacterial communities in streams are exposed to a variety of DOC compounds in the form of leaf litter, DOC leached from soil, groundwater inputs, as well as in-stream primary production (Trumbore et al., 1992; Allan and Castillo, 2001). The latter DOC source may be generally limited in streams because benthic algae are often light limited from shading and turbidity (Vannote et al. 1980). Such diversity in DOC sources suggests the possibility of niche partitioning among stream bacteria with respect to utilization of such varied DOC sources (Koetsier et al., 1997; Olapade and Leff, 2005; Singer et al., 2010; Perryman et al., 2011).

In fact, several studies have investigated bacterial structure and function with respect to heterogeneity of DOC in streams. For example, in the study conducted by Olapade and Leff (2005), treatments with different DOC sources such as glucose, leaf leachates and algae exudates led to differences in abundance among bacterial taxa. While such studies have considered differences in structural aspects; bacterial functions, such as denitrification, have also been observed to be affected by heterogeneity of DOC sources (Schipper et al., 1994; Pfenning and McMohan, 1996; Hill and Cardaci, 2004) and denitrifiers have been demonstrated to utilize a diverse array of organic compounds (Zumft, 1997). Denitrification is an important function carried out in stream sediments by denitrifying bacteria, preventing accumulation of nitrate in these ecosystems (Mul holland et al., 2008) and requiring DOC for the process. Hence, indications that DOC quality may
affect denitrification rates and determine denitrifier community structure necessitates further investigations on the topic.

In contrast to streams, in lakes, phytoplankton exudates are an important source of DOC for bacterioplankton, especially during the occurrence of blooms. Bacterial communities play a major role in uptake of algal exudates which fulfills a considerable portion of their carbon demand (Baines and Pace, 1991; Ducklow and Carlson, 1992). For example, a study done by Desey et al. (2002) demonstrated that 77% of the total algal exudates were taken up by bacteria which accounted for 22% of their carbon demand. Even such relatively homogeneous source of DOC (compared to stream ecosystems) such as algal photosynthetic exudates, leads to differences in bacterial communities in the form of particle-attached and free-living fractions, especially during an algal bloom (Selje and Simon, 2003; Rink et al., 2007). Differences have also been observed between carbon uptake and bacterial enzymatic activities of particle-attached and free-living fractions (Unanue et al. 1998; Riemann et al. 2000; Lehmann and O’ Connel, 2002). Other studies, on the other hand, have demonstrated no such differences between the two fractions (Riemann and Winding, 2001; Worm et al., 2001). Such observational differences among studies paves the way to further investigations on whether presence of a dominant DOC source, such as algal blooms, is actually important in determining bacterial responses.
Link between bacterial community structure and DOC utilization

The linkage between bacterial structure and function, especially related to carbon utilization, is a highly debated topic in microbial ecology (Philipot and Hallin, 2005; Judd et al., 2006; Fornara and Tilman, 2009; Theuerl and Buscot, 2010) and no consensus has been reached yet on the presence or absence of such relationships. While some studies have demonstrated that community structure is independent of carbon use (Reinthaler et al., 2005; Bell et al., 2009; Frossard et al., 2011), others have shown that the capability of bacteria to use one form of carbon over the other can be determined by its structure (Kritzberg et al., 2006; Murray et al., 2007; Obernosterer et al., 2010). Such structure-function relationships in bacterial communities seem to be dependent on the ecosystem. For example, while Rich et al. (2003) demonstrated that differences in denitrifier community structure followed the same pattern in denitrification rates when comparing forest and meadow soils, Rich and Myrold (2004) found that community structure and function (denitrification rates) were uncoupled in agroecosystems when wet soils and creek sediments were compared.

Absence of linkages between community structure and function may result from two possible causes: the ability of different bacterial communities to perform similar functions, also known as functional redundancy, (Allison and Martiny, 2008) and the capability of the same bacterial communities to perform different functions (Peter et al., 2011). In either case, absence of structure-function coupling can lead to ecosystem resilience in the event of disturbance (Bowen et al., 2011). Therefore, it is important to
study both structural and functional responses of bacteria to DOC, in order to understand its effect on ecosystem stability.

Organizational scheme of the Dissertation

My dissertation aims to understand the role played by DOC sources in structuring bacterial communities and determining their function in aquatic ecosystems. The link between bacterial community structure and function will also be discussed. The dissertation is comprised of three chapters describing the research done on the topic, followed by a synthesis of major findings and conclusions drawn from them.

- Chapter 2 examines the effect of presence and absence of a dominant DOC source, in other words a cyanobacterial bloom in a lake ecosystem (Lake Erie), on bacterial community structure and function, namely, heterotrophic productivity and carbon uptake.
- In Chapter 3, the effect of quality and molecular heterogeneity of DOC source on stream bacterial community structure and denitrification potential are examined.
- In Chapter 4, I investigate whether bacterial community structure and function vary based on predominance of allochthonous and autochthonous carbon sources along a riverine-lacustrine gradient.
- Chapter 5 summarizes and compares all observations and explores implications of the study in a broader context.
Although many studies in the past have examined bacterial responses to DOC in aquatic ecosystems, this work fills several significant knowledge gaps. First, many of the studies conducted previously have examined bacterial community structure or function, rather than examining both simultaneously. In each chapter, I examined both structure and function. Lack of significant knowledge on structure-function linkage is especially true in relation to phytoplankton-bacterial coupling and has been addressed in chapter 2. Second, the information gathered in prior studies about structure is limited; in contrast, in Chapter 3 both taxonomically relevant genes (16S rRNA genes) and functional genes (nosZ which encodes for nitrous oxide reductase that performs a key step in denitrification) were examined. Third, although the effects of different DOC sources on some processes, like denitrification, have been examined previously, impacts of molecular heterogeneity mostly remain unexplored (Chapter 3). Fourth, prior studies on DOC-bacteria relationships have generally examined reservoirs/lakes and rivers separately; in Chapter 4 I examine these relationships along a longitudinal gradient from a river to a reservoir. Finally, while most studies have compared bacterial response to DOC quality among broad classes, the study takes into consideration the more detailed differences in chemical composition within each broad category.
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CHAPTER 2

Changes in structure and function of bacterial assemblages

in Lake Erie during *Microcystis* blooms

ABSTRACT

Bacterial assemblage structure and function were examined in three highly productive sites in the Western Basin of Lake Erie experiencing recurring *Microcystis* blooms. Samples were collected at five different times during the bloom from two sites in Maumee Bay (sites 1 and 2) and one in Sandusky Bay (site 3). Community structure, abundance, productivity and algal exudate (photosynthate) uptake of particle-attached and free-living bacteria were examined. Abundance of free-living bacteria was generally higher than that of particle-attached fraction, with few exceptions. Particle-attached bacteria had higher per cell productivity and photosynthate uptake rate compared to free-living bacteria. Bacterial community structure of each fraction, examined using

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denaturing gradient gel electrophoresis of 16S rRNA gene polymerase chain reaction products changed over the sampling period. Community structure of particle-attached and free-living fractions, with a single exception, differed from each other on a given date. However, similarities were observed between particle-attached and free-living bacteria from different dates within each site, suggesting the presence of generalist, rather than particle-specialist, bacteria. Bacterial assemblage structure was not related to function since functional differences were observed irrespective of differences in structure and vice versa. Overall, highly productive systems in Lake Erie demonstrating Microcystis blooms benefitted the particle-attached community more than the free-living fraction, in terms of bacterial productivity and photosynthate uptake, and led to a clear difference between the two fractions in community structure at a particular time point. However, structure was not related to function. Differences between particle-attached and free-living bacterial communities even in the absence of Microcystis suggests that such functional advantage is not attributable to co-occurrence of specific taxa with the cyanobacteria but is rather a result of association with particles under such highly productive conditions.

INTRODUCTION

Algal blooms are accompanied by associated particle-attached bacteria (Riemann and Winding, 2001). These associations occur as bacteria take advantage of labile dissolved organic carbon (DOC) released by algae (Cole et al., 1988; Smith et al., 1995;
Grossart and Simon, 1998). Production of exudates by algae occurs when photosynthesis exceeds assimilation and excess photosynthate is released (Fogg, 1983) providing readily available carbon for heterotrophic bacteria. In turn, this labile DOC from algal exudates impacts bacterial community composition and function (Pinhassi et al., 2004; Passow et al., 2007; Rink et al., 2007).

One manifestation of the impacts of algal exudates is differences between free-living and particle-attached fractions of the bacterial assemblage. Some previous studies have demonstrated that composition of the two fractions varies over the course of an algal bloom leading to clear differences between attached and free-living bacterial assemblages (Fandino et al., 2001; Selje and Simon, 2003; Rink et al., 2007). Conversely, other studies have not found such differences between the two fractions, contradicting the idea of phylogenetically distinct ‘particle specialists’ (Hollibaugh et al., 2000; Riemann and Winding, 2001; Ghiglione et al., 2009). Generally, differences (or the lack thereof) between the structure of free-living and particle-attached fractions appear to be dependent on the study site (Hollibaugh et al., 2000; Garneau et al., 2009).

The impact of differences in bacterial assemblage structure between free-living and particle-attached fractions on bacterial function is not well understood. In terms of metabolic differences, some studies have shown that the two fractions differ in uptake of algal exudates (Unanue et al., 1998; Riemann et al., 2000), while another study found no such difference (Worm et al., 2001). Contradictory evidence has also been observed in studies related to activity of the two bacterial fractions. On the one hand, while some
studies have demonstrated particle-attached bacteria are more active than free-living bacteria (Grossart et al., 2007; Taylor et al. 2009), others have found the opposite trend (Alldredge et al., 1986; Martinez et al., 1996). In general, it is not known whether such functional differences are related to structure.

DOC uptake by bacteria depends on their physiological requirements and enzyme production which, in turn, depends on community structure (Fandino et al., 2001). Thus, bacterial community structure can influence the rate of carbon uptake and productivity (Chróst and Rai, 1993; Rath et al., 1993), but functional differences can also occur because of changes in metabolic activity of the existing assemblage (Riemann et al., 2000). In any case, differences in structure and function between attached and free-living bacterial assemblages may result in differential transfer of carbon to higher trophic levels (Lehman and O’Connell, 2002).

Carbon transfer through the microbial loop plays an important role in the Great Lakes (Fahnenstiel et al. 1998; Heath et al., 2003), like other aquatic ecosystems (e.g., Azam et al. 1993). Algae-bacteria interactions also play a key role in the microbial loop (Carrillo et al., 2006) and functional differences between the two bacterial fractions, in terms of carbon uptake and productivity, are likely to affect carbon transfer to higher trophic levels. For example, predation pressure on particle-attached bacteria may be lower than that of free-living bacteria during a cyanobacterial bloom as most toxic cyanobacteria are largely inedible (Vanderploeg et al., 2001). Hence, in such cases, increased productivity and carbon uptake by the particle-attached fraction when
compared to the free-living bacteria will result in less efficient mobilization of carbon from algal exudates to higher trophic levels.

*Microcystis* is a suitable habitat for bacteria (Worm and Sondergaard, 1998), and *Microcystis* blooms have occurred frequently in Lake Erie, especially in summer, since the mid 1990s (Rinta-Kanto et al., 2005). Toxicity and large colony size makes *Microcystis*, and hence the attached heterotrophic bacteria, inedible to grazers (Fulton and Paerl, 1987a, b). In spite of the prevalence of *Microcystis* blooms and the importance of heterotrophic bacteria to the carbon cycle in Lake Erie, previous studies have not examined the response of bacterial communities to such a dominant DOC source. Furthermore, it is not known if such responses are determined by bacterial community structure or whether they are independent of structural make-up. We hypothesize that if presence of *Microcystis* is particularly instrumental in determining bacterial responses, then structural and functional differences between particle-attached and free-living fractions will not be observed in the absence of the cyanobacteria. We also predict that functional differences between the two bacterial fractions will be linked to differences in community structure.

Understanding the role of *Microcystis* in determining bacterial productivity and carbon transfer to bacterial communities will reveal potential impacts on the contribution of autochthonous carbon from the recurring blooms in meeting carbon demands of higher trophic levels in these highly productive sites of Lake Erie. In this study, structure, abundance, productivity and carbon uptake of particle-associated and free-living bacteria
during a *Microcystis* bloom in Lake Erie were examined. Bacterial assemblage structure was examined relative to function to infer whether functional differences over time are related to differences in structure.

**MATERIALS AND METHODS:**

*Sample collection and study site*

Water samples were collected with an integrated tube sampler (3-m long, ~ 3-cm inner diameter PVC pipe) from 1m below the surface from two sites in Maumee Bay near the river outflow (Site 1; 41° 43’ N, 83° 22’ W and Site 2; 41° 43’ N and 83° 17’ W) and one site in Sandusky Bay east of the Sandusky River discharge (Site 3; 41° 28’ N and 82° 37’ W). Samples were collected from each site on 06/01/09, 07/14/09, 08/11/09, 10/01/09 and 10/20/09 and were transported on ice reaching the laboratory within 6 hours of collection. Sandusky and Maumee Bays are parts of two agriculturally impacted watersheds and demonstrated a dominance of *Microcystis* during this study. While *Microcystis* is usually dominant in Maumee Bay, some parts of Sandusky Bay experience blooms of other cyanobacteria (Rinta-Kanto et al., 2005) revealing differences among these areas which may impact bacterial responses. In Maumee Bay, site 1 was upstream of site 2 and because, upon entering the bay, cyanobacteria will be alleviated from light limitation (Conroy et al., 2007), these sites were expected to differ in timing of the bloom.
Laboratory processing

In the laboratory, samples in triplicate were filtered through GF/F filters (0.7-µm nominal pore diameter, Whatman) and total dissolved organic carbon (TDOC) concentrations were determined using a TOC5000 analyzer (Shimadzu Scientific Instruments). Whole water samples (one sample per site) were frozen for analysis of total phosphorus (TP) and total Kjeldahl nitrogen (TKN) and were later digested for analysis. Samples filtered through 0.22-µm pore-size filters (Whatman, one sample per site) were used to determine soluble reactive phosphorus (SRP), nitrate and nitrite concentrations using a Lachat QuickChem flow injection analyzer 8000 series (Hach).

Algal characteristics, namely, chlorophyll a (chl a) concentration and Microcystis biomass, were determined from single samples collected at each site on each date. Samples for chl a measurements were filtered through GF/F filters (0.7-µm nominal pore diameter, Whatman) and frozen for later analysis. Subsequently, spectrophotometric analysis was done using a Shimadzu UV-1800 spectrophotometer (Shimadzu Scientific Instruments). Subsamples for examination of phytoplankton community composition were preserved in Lugol’s iodine solution, observed under 400X using an inverted microscope (Wild-Heerbrugg M40) and algae as well as cyanobacteria, including Microcystis, were enumerated. Biovolume was calculated from cell numbers and average cell size and used to calculate biomass using the nominal density of water.
as a conversion factor as has been previously done for Lake Erie phytoplankton (Conroy et al. 2005).

Primary productivity was calculated for one sample per site per date from whole water incubated with $^{14}$C-NaHCO$_3$ (20 µCi) for an hour using a photosynthetron. Post-incubation, whole water samples were filtered on 1-µm pore-size filters and sparged in an acidic environment to remove residual inorganic carbon. Radioactivity was measured by liquid scintillation in a Beckman LS6800 liquid scintillation counter (Beckman Inc.).

Samples for bacterial enumeration (three samples per site and per date) were divided into two fractions. To dislodge bacteria from particles, one fraction was treated with 10% methanol (v/v), incubated at 35°C for 15 minutes, and sonicated for 5 minutes using a Branson 2210 sonicator (Branson) following a protocol modified from Lunau et al. (2005). The methanol-treated and sonicated fraction was preserved with 8% paraformaldehyde and included both particle-attached and free-living bacteria. The other fraction (the free-living or planktonic bacteria) was filtered through 1-µm pore-size polycarbonate filters (Poretics) to remove large particles and preserved as above. Bacteria in both fractions were enumerated after filtration on to 0.22-µm pore-size black polycarbonate filters (Poretics) and staining with 4',6-diamidino-2-phenylindole (DAPI) for 3 minutes. Bacteria in 10 fields per slide were counted via epifluorescence microscopy. Free-living bacterial numbers were subtracted from total counts to calculate particle-attached bacterial abundance.
Bacterial heterotrophic productivity was determined from triplicate samples by measuring $^3$H-leucine incorporation (Jorgensen 1992). Assays were preformed alongside formalin-fixed controls. Each sample was amended with $^3$H-leucine (New England Nuclear Corporation – DuPont, 50 Ci/mmol specific activity) and incubated for 1 hour. Precipitated macromolecules were rinsed by three washes of 5% trichloroacetic acid. Samples were filtered through 1-µm pore-size filters (polycarbonate, Poretics) and used to estimate productivity of the particle-attached fraction. Bacteria in the filtrate were collected on 0.22 µm pore-size filters (polycarbonate, Poretics) and used to determine productivity of free-living bacteria. $^3$H dpm of each filter was determined by liquid scintillation in a Beckman LS6800 liquid scintillation counter (Beckman Inc.). Formalin-fixed controls were used to correct for background radioactivity. Conversion factors for bacterial carbon production were determined using the derivative method of Jorgensen (1992) and Simon and Azam (1989). Bacterial heterotrophic productivity per cell was calculated by dividing productivity by the average number of cells.

Uptake of nascent photosynthate by particle-attached and free-living bacteria was measured on triplicate samples from each site on each date by stepwise filtration after incubation of samples with $^{14}$C-NaHCO$_3$ (20 µCi) for 6 hours under the same light source used to measure primary productivity. Controls were incubated in the dark. After incubation, samples were filtered through 1-µm pore-size polycarbonate filters (Poretics) to separate the particle-attached fraction from the free-living fraction and filtrate (comprising the free-living fraction) was passed through 0.22-µm pore-size polycarbonate filters (Poretics). The filters were rinsed thrice with deionized water and
placed in a strongly acidic atmosphere to sparge residual carbon dioxide. Radioactivity was measured by liquid scintillation in a Beckman LS6800 liquid scintillation counter (Beckman Inc.). Carbon uptake by free-living bacteria and particle-attached fractions was calculated as in Descy et al. (2002) and divided by the average number of cells to obtain per cell carbon uptake. All values were corrected for background radioactivity by subtracting control values.

For extraction of DNA from particle-attached and free-living bacterial fractions, three samples from each site and date were filtered on 1-µm and 0.22-µm pore-size polycarbonate filters (Poretics), respectively. Filters were frozen (-80 °C) until DNA was extracted using the PowerSoil DNA extraction kit (MoBio Laboratories) following the manufacturer’s protocol. PCR was performed using 968F+GC clamp (5´GCCCGCCGCAGCGCGGCGGACGGGGGGAACGCGAAGACCTTAC, 3´; Heuer and Smalla, 1997) and 1392 R (5´-ACGGCGGTGTGTRC, 3´; Brosius et al. 1981) primers. Reactions were carried out for 30 cycles in a PTC-100 programmable thermal controller (M.J. Research Inc.). Each 25 µl reaction mixture contained 15.25 µl of water, 5 µl of 5X green Go Taq Flexi buffer, 2 µl of 25 mM MgCl₂, 0.5 µl of 10 mM PCR nucleotide mix containing 10 mM of each dNTP, 0.125 µl each of primer, 2 µl of DNA template, and 0.125 µl of DNA polymerase. Reaction conditions followed the protocol of Heuer et al. (1997) as modified by van Dillewijn et al. (2002). Positive (Pseudomonas aeruginosa DNA, American Type Culture Collection, BAA-47) and negative controls were included in each PCR run.
PCR products were run on a denaturing gradient gel (Muyzer and Waal, 1995) using the DCode system (Bio-Rad Laboratories). Amplified products were loaded in 6% polyacrylamide gels run in TAE (Tris-acetate-ethyldiaminetetraacetic acid) buffer. The denaturing gradient used ranged between 30% and 60% and electrophoresis was carried out at 150 V for 6 hours. A 1 kb ladder was loaded in one or two of the wells and a positive control (*Pseudomonas aeruginosa*) in two others. Gels were stained with Sybr Gold stain (1:10,000 dilution, Bio Rad Laboratories) for 45 minutes and images were captured with Gel doc imaging (Bio-Rad Laboratories).

**Statistical analyses**

To determine differences among dates, sites, fractions (particle-attached and free-living) and interactions among these factors, data, except DGGE images, were analyzed by three-way analyses of variance (ANOVA) in JMP 8. Multiple linear regression with stepwise forward and backward selection (using JMP 8) was used to determine the relationship between the measurements of bacterial functional parameters and *Microcystis* biomass, chlorophyll *a* concentration and primary productivity as well as the other environmental variables like nutrient and DOC concentrations. The values to enter and leave the analyses were 0.100 and 0.05, respectively.

For analysis of DGGE profiles, unweighted pair group method and arithmetic average (UPGMA) was done using R to create a binary matrix for cluster analysis. Cluster analysis was performed to derive linkage dendograms which in turn were used to
depict similarity between free-living and particle-attached bacterial community structure among dates at every site. The number of bands from the DGGE gels were compared among site, date, fractions as well as their interaction by three way ANOVA followed by post-hoc Tukey test using JMP 8 statistical software.

RESULTS:

Occurrence of Microcystis (Fig. 2.1A) was observed on most sampling dates with a few exceptions. The upstream site in Maumee Bay (site 1) demonstrated presence of Microcystis throughout the sampling period whereas it was not observed until a later date (11-August) in the downstream site (site 2). In the Sandusky Bay site (site 3) Microcystis was absent only on the first sampling date and observed on all others. Presence of other primary producers (Fig. 2.1B) as well as primary productivity (Fig. 2.1C) was detectable even in the absence of Microcystis.

DOC concentrations (Fig. 2.2) were significantly different among sites (P<0.05, df =2, F= 217) and dates (P<0.05, df = 3, F = 246), as well as a significant date and site interaction (P<0.05, df = 6, F = 21). Except at site 2, DOC concentration decreased from 14-July through 20-October. Concentration in Site 2 increased from 14-July to 11-Aug and then declined. On each sampling date, site 1, the most upstream site in Maumee Bay, consistently had the highest DOC concentration followed by site 2, which was further downstream. Site 3, in Sandusky Bay, had the lowest DOC concentrations.
Fig. 2.1: A) Biomass of *Microcystis* spp, B) Chlorophyll *a* concentrations and C) Primary productivity of the sites sampled in Maumee (sites 1 and 2) and Sandusky (site 3) Bays collected on the given dates, in the year 2009, from a depth of 1m below the water surface. Each bar represents a single sample.
Fig. 2.2: Total dissolved organic carbon (TDOC) concentrations of water samples collected from sites 1 (Maumee Bay), 2 (Maumee Bay) and 3 (Sandusky Bay) on the respective dates in the year 2009. Values are means and standard errors from triplicate samples. Data from 1-June, 2009 is not available.
Bacterial abundance (Fig 2.3) differed significantly between the two fractions (P<0.05, df =1, F = 37.51) and among dates (P<0.05, df = 4, F = 9). The fraction by date interaction (P<0.05, df = 4, F = 5) and date by site by fraction interaction were also significant (P<0.05, df = 8, F = 6). Differences among sites, as well as the site by date and fraction by site interaction, were not significant. Abundance of free-living bacteria was generally higher than or similar to particle-attached bacteria except on 1-June at sites 2 and 3, when particle-attached bacteria were more abundant. There were also changes over time; abundance of free-living bacteria decreased between 1-June and 20-October in sites 1 and 2, and between 14-July and 20-October in site 3. Particle-attached bacterial numbers decreased significantly between 14-July and 11-August in all sites. Henceforth, there were no significant differences among dates in particle-attached bacterial abundance in sites 1 and 3 while at site 2 numbers increased significantly again on 20-October, 2009.

Cell-specific bacterial secondary productivity (Fig. 2.4) differed significantly between free-living and particle attached fractions (P<0.05, df = 1, F = 24). In addition, there were significant differences in productivity among dates (P<0.05, df = 4, F = 8) and sites (P<0.05, df = 2, F =11) and the date by site interaction was significant (P<0.05, df = 8, F = 24,). Other interactions were not statistically significant. Particle-attached bacteria had significantly higher per cell productivity compared to free-living bacteria, in most cases, except on 14-July at sites 1 and 2, on 20-October at site 2 and on 1-June at site 3 when productivity of free-living bacteria was higher than particle-attached. Differences among sites were also observed: bacterial productivity at site 2 was significantly lower
than at sites 1 and 3. Patterns of temporal change varied among sites. At sites 1 and 2, in Maumee Bay, there was a significant decrease in particle-attached bacterial productivity between 1-June and 14-July followed by an increase on 20-October only in site 1. At site 3, in Sandusky Bay, particle-attached bacterial productivity on 20-October was higher compared to the other dates.

Uptake of carbon released by primary producers (on a per cell basis) was significantly different between the two bacterial fractions (P<0.05, df = 1, F = 6, Fig. 2.5); uptake rates of particle-attached bacteria were higher than free-living bacteria. Uptake of photosynthate by heterotrophic bacteria was not significantly different among dates and sites, and interactions were not significant. As with production, there was a peak in bacterial photosynthate uptake on 20-October at site 3. At other sites, uptake by attached bacteria peaked in 11-August.

Multiple linear regression was used to examine relationships among the bacterial responses and environmental variables. These variables included DOC and nutrient concentration along with algal variables such as chl a concentration, *Microcystis* biomass and primary productivity. Bacterial heterotrophic productivity was most strongly related to primary productivity ($R^2 = 0.31$, $R^2$ adj= 0.29, P<0.05) and inclusion of other variables did not significantly improve this regression. In contrast, bacterial abundance and photosynthate uptake were not related to the algal or environmental variables.
Fig. 2.3: Abundance of free-living and particle-attached bacteria (based on DAPI staining) in: A) Site 1 (Maumee Bay), B) Site 2 (Maumee Bay) and C) Site 3 (Sandusky Bay) from 1-June, 2009 to 21-Oct, 2009. Values are means and standard errors from triplicate samples.
Fig.2. 4: Heterotrophic productivity of the free-living and particle-attached fractions of bacteria in A) Site 1 (Maumee Bay), B) Site 2 (Maumee Bay) and C) Site 3 (Sandusky Bay) in Lake Erie on the respective dates. Values are means and standard errors obtained from triplicate samples.
**A**

- Free-living
- Particle-attached

**B**

**C**
Fig.2.5: Uptake of $^{14}$C-labeled carbon by free-living and particle attached fractions after NaH $^{14}$CO$_3$ addition followed by 6 hours of incubation under constant light conditions. A) Site 1 (Maumee Bay), B) Site 2 (Maumee Bay) and C) Site 3 (Sandusky Bay). Values are means and standard errors from triplicate samples. Data from 1-Jun, 2009 is not available.
Bacterial photosynthate uptake (mmolC/cell/hr) $\times 10^{-12}$

**A**
- Free-living
- Particle-attached

**B**

**C**
DGGE revealed differences in particle-attached and free-living bacterial community structure which was not linked to the presence or absence of *Microcystis* (Fig. 2.6). At site 1, the upstream site in Maumee Bay, the structure of particle-attached and free-living bacteria assemblages were similar on 1-June, 2009 while the two communities were different thereafter. Also at this site, structure of the particle-attached assemblage changed over time (1-June differed from 11-August and 1-October differed from 20-October). Free-living bacteria at site 1 also changed over time; 1-June and 11-August communities differed from each other and from 1-October and 20-October communities, the latter two dates having similarities in community structure.

At site 2, further downstream in Maumee Bay, particle-attached and free-living bacterial community structure from a given date differed from each other throughout. Also, at this site, while differences were observed among particle-attached communities on all dates, free-living bacteria from 11-August and 1-October were similar, but differed on the other dates. Such differences in structure were also observed in site 3 barring the similarity between particle-attached bacteria on 1-June and 20-October.

Also noticeable in all the sites was the similarity of particle-attached bacteria from one date to the free-living fraction from another. For example, in site 1, free-living bacteria from 11-August were similar to the particle-attached fraction from 20-October, 2009. Similarly in site 2, the particle-attached bacteria from 1-October was similar to free-living fraction from 20-October, 2009. The same trend in similarity between particle-attached and free-living bacterial fractions was observed in site 3, as in site 2.
Number of bands from DGGE (hereafter referred to as ribotypes) was significantly different between the particle-attached and free-living fractions at all sites (P<0.05, df = 2, F = 1, Fig. 2.7). Ribotype number differed significantly among dates (P<0.05, df = 3, F = 3) and sites (P<0.05, df = 2, F = 33); the interaction among site and fraction was also significant (P<0.05, df = 6, F = 18). At site 1, particle-attached communities had greater numbers of ribotypes than free-living bacteria (except on 11-August, 2009 when they were similar). On the other hand, at sites 2 and 3, the number of ribotypes from the free-living fraction was higher than that of the particle-attached fraction. The only exceptions to this were site 2 on 20-October when the number ribotypes of the two fractions were similar, and site 3 on 1-June when the number was higher for the particle-attached fraction. In general, the number of ribotypes at site 1 was significantly higher than at sites 2 and 3. There were also temporal changes; ribotype number decreased significantly over time for site 1, while in site 3 the number of bands increased from 1-Jun to 11-August followed by a significant decrease from 1-October to 20-October. In site 2, the number of bands decreased from 1-June to 11-August and again from 1-October to 20-October, 2009. Overall, there was a decrease in the number of ribotypes for each fraction over time, with some exceptions, and differences in ribotype number between the free-living and particle-attached bacteria were site dependent.
Fig. 2.6: Cluster dendograms of DGGE images from UPGMA for samples collected on different dates in Sites A) 1, B) 2 and C) 3. Data for 14-July, 2009 is not available. PA indicates particle-attached and FL indicates free-living bacteria.
Fig 2.7: Average number of bands in A) site 1, B) site 2 and C) site 3 obtained from DGGE images on different dates. Data for 14-July-2009 is not available. Values are means and standard errors.
A

Free-living

Particle-attached

Number of bands

1-Jun 11-Aug 1-Oct 20-Oct

B

Number of bands

1-Jun 11-Aug 1-Oct 20-Oct

C

Number of bands

1-Jun 11-Aug 1-Oct 20-Oct

Date

55
**DISCUSSION:**

In this study, structure and function of bacterial assemblages were studied over time, in three highly productive sites in Lake Erie I the presence and absence *Microcystis*. Changes in both structure and function were observed but structure did not always correlate with functional differences. While some of these changes, like bacterial heterotrophic productivity, were correlated with characteristics of the primary producers, others like bacterial abundance and carbon uptake were not. Furthermore, there were significant differences between the particle-attached and free-living bacteria in most cases. While particle-attached communities were less abundant, they had higher per cell secondary productivity and photosynthate uptake rates. This suggests that colonization of particles can be particularly advantageous to bacteria. The study also suggests that bacterial taxa are not specialists, with regards to particle colonization in these highly productive systems, even in the presence of mucilaginous *Microcystis* colonies.

Considered together these observations suggest that in the ecosystems studied, structure does not necessarily relate to function, particle colonization is not directly influenced by presence or absence of *Microcystis*, but such associations do provide a functional advantage to bacteria.

Bacterial communities were differentially related to algal variables such that bacterial productivity demonstrated a positive relationship with primary productivity, while photosynthate uptake and abundance were not related to any of the algal variables. Consistent with our findings, a positive correlation between algal biomass or primary
production and bacterial heterotrophic productivity has been observed in other studies of freshwater systems (White et al. 1991; Auer and Powell, 2004; Kritzberg et al. 2005; Ortega-Retuerta et al., 2008; Fouilland and Mostajir, 2010). On the other hand, some studies have not found relationships between primary production and bacterial secondary productivity (Pace and Cole 1994; Moran et al., 2002). Heath and Munawar (2004) suggest that such a correlation is a function of the physiological condition of the primary producers. The importance of physiological state of both algae and bacteria may be one explanation for the disparity among studies. Dependence of bacterial productivity on physiological state may also explain the lower productivity rates in the particle-attached fraction, than in the free-living counterpart, on 14-July in sites 1 and 2, in spite of detectable primary productivity rates. In addition, bacterial productivity in some systems may be uncoupled from primary production due to dependence on allochthonous carbon sources (Jansson et al. 2000).

Surprisingly, bacterial uptake of algal exudates was not significantly related with the algal variables measured. Possibly photosynthate uptake rates are an inherent function of bacterial physiological requirements that do not change with productivity or biomass of primary producers. Uptake rates of dissolved organic carbon is dependent on enzymatic activities (Arnosti et al., 2005; Arnosti, 2011) which in turn is related to the physiological status of bacteria (Agis et al., 1998; Arnosti et al., 2005) or bacterial community composition (Arnosti, 2011). Since, in this case, community structure was not coupled to function, the alternative explanation is more probable.
Similarly, bacterial abundance was independent of the measured algal variables. Previous studies on lake ecosystems have shown that abiotic factors, like temperature and nutrient concentrations, and biotic factors, like predator abundance influence bacterial abundance more than variables related to algal biomass, like chlorophyll a concentrations (Le et al., 1994; Coveney and Wetzel, 1995; Gurung and Urabe, 1999, Staroscik and Smith, 2004; Gurung et al. 2010). However, in this study no significant relation of bacterial abundance was observed with nutrient or DOC concentration as well.

Particle-attached and free-living bacterial assemblages differed in their function. Particle-attached bacteria generally had higher per cell productivity and rates of photosynthate uptake but lower abundance compared to free-living bacteria. Results from prior studies that have compared attached and free-living bacteria differ. In some cases, productivity of attached bacteria was higher than their free-living counterparts (Crump et al., 1998; Riemann et al., 2000; Luef et al., 2007). In contrast, Karrasch et al. (2003) did not find a difference between the two fractions in terms of productivity and extracellular enzyme production. Differences in outcome among studies may also be attributed to physiological properties of bacteria or primary producers, as discussed before. In spite of different outcomes with respect to bacterial functions, abundance of free-living bacteria has been observed to be higher than that of particle-attached bacteria in most studies (Rink et al., 2007; Ghiglione et al., 2009; Lapoussière et al., 2011), which is consistent with our findings.
Differences in function between free-living and particle-attached fractions were observed even in the absence of Microcystis, while similarities were seen in spite of its presence. For example, bacterial heterotrophic productivity of free-living and particle-attached fractions was significantly different in site 2 on 1–June and 14–July even though Microcystis biomass was below detection. On the other hand, bacterial photosynthate uptake was similar between the two fractions in site 1 on 11–August, albeit the presence of Microcystis. As has been mentioned before, results from multiple regresional analyses also demonstrate that none of the variables were related to Microcystis biomass. Rather, bacterial heterotrophic productivity demonstrated a relationship with primary productivity in general. These observations taken together indicate that association with particles, rather than the presence or absence of a certain kind of primary producer, are more instrumental in determining bacterial response in a highly productive system. However, once such associations occur, they can be advantageous to bacteria.

In addition, temporal changes in bacterial variables over time were not related to the presence or absence of Microcystis. For example, a decrease in bacterial abundance was observed in site 2 between 14–July, when Microcystis was absent, and 11–August, 2009, when it was detectable; but in site 3, demonstrating similar abundance as site 2, the same trend of decrease was evident even in the presence of Microcystis on both dates. Similarly, trends of change in bacterial productivity were unrelated to presence or absence of the cyanobacteria. For example in site 2, bacterial productivity decreased from 1–June to 14–July, 2009 but Microcystis was not detectable on both occasions. Also, in site 3, bacterial productivity on 1–June, when Microcystis was not detectable, was
similar to other dates (except 20- October) on which the cyanobacteria was present. The same applied for bacterial photosynthate uptake that was similar among all sampling dates irrespective of the presence or absence of Microcystis.

Free-living and particle-attached bacterial assemblage structure, as determined from DGGE bands, differed at each sampling time point, with the exception of a single date at one of the sites. Several studies have demonstrated such differences in community structure between the two fractions during phytoplankton blooms in marine systems (Moeseneder et al., 2001; Fandino et al., 2001; Grossart et al., 2007). Generally, in our study, structural differences were observed throughout the study period. However, similarities in community structure between the free-living and particle-attached fractions were observed at different time points. This suggests that, in Lake Erie, particles are colonized by generalists rather than particle-specialists as has been suggested by studies in other ecosystems (Riemann et al., 2000; Ghiglione et al., 2007). Some other studies have suggested that, such similarities may be observed because, particle-attached and free-living fractions equilibrate between the particles and the water column (Riemann and Winding, 2001; Worm et al., 2001; Ghiglione et al., 2009). Methods used for examining bacterial community structure differ among these studies and an assumption of DGGE is that bands that migrate the same distance are the same phylotypes and represent a single taxon (Fandino et al., 2001). It is also likely that some of the bands from the DGGE gels include cyanobacteria in the particle-attached fraction, but most of the variation in band number and position is accounted for by heterotrophic bacteria.
Bacterial community structure was not always related to functional differences. Even when there were similarities in community structure, differences between the particle-attached and free-living fractions productivity and abundance were observed. For example, at site 1 on 1-June, free-living bacterial abundance was higher and secondary productivity was lower in comparison to particle-attached bacteria even though the community structure of both fractions was similar. Even within each fraction, similarities or differences in community structure also did not translate to differences in function. For example, although free-living communities in site 1 differed between 11-August and 1-October, there was no difference in bacterial heterotrophic productivity. Thus, community structure does not always predict functional differences between the fractions.

Overall, the results indicate that bacterial structural and functional responses in a highly productive ecosystem may not be directly influenced by a dominant primary producer, even though certain aspects of primary production in general may be linked to some of the responses. For example, in the current study, attachment to particles led to differences in bacterial structure and function, compared to the free-living counterparts, irrespective of the presence of *Microcystis*. While bacterial heterotrophic productivity was directly related to primary production, others like bacterial abundance and uptake of algal exudates were not. Although both structural and functional differences resulted from attachment to particles, structure was not a good predictor of functional differences in these ecosystems.
Most importantly, association with particles is beneficial for bacterial function, and if such particles are inaccessible to predators, this may have negative consequences on autochthonous carbon transfer to higher trophic levels through bacteria. In this study, attachment to particles led to higher productivity and uptake of algae exudate in bacteria, compared to the free-living fraction. Mucilagenous colonies of *Microcystis* can not only serve as potential sites of attachment, but also as a source of autochthonous carbon for bacteria. Based on observations from the study, productivity and algal exudate uptake rates of bacteria attached to *Microcystis* will then be higher than in the free-living fraction. The cyanobacteria being toxic and inedible, such highly productive bacteria will then remain inaccessible to predators, thereby having a negative effect on carbon transfer through the microbial loop in these ecosystems.

**ACKNOWLEDGEMENT:**

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CHAPTER 3

Effect of molecular diversity and complexity of dissolved organic carbon sources on community structure and denitrification potential of stream bacteria

ABSTRACT

Bacterial response to broad classes of dissolved organic carbon (DOC) has been examined in prior studies, but, in the present study, the effect of molecular heterogeneity within each broad class, on bacterial community structure and function was examined. In this study, aerobic and anaerobic microcosms containing stream bacterial communities grown on artificial substrates were amended with simple and complex DOC compounds individually and in mixtures. Amendments were broadly divided into chemically defined and undefined categories. The chemically defined compounds were labile (glucose, glycine and acetate) and recalcitrant (coumaric, syringic and vanillic acids) compounds while the chemically undefined were algae (Chlamydomonas, Chlorella and Synedra) exudates and leaf (red oak, witch hazel and corn) leachates. Ion exchange
chromatography was used to fractionate DOC from each of the undefined sources into humic and non-humic fractions. Leaf leachates had higher percentage of aquatic humic substance (AHS, average 33 ± 2.43% of total DOC) compared to algae exudates (average 14 ± 1.82% of total DOC). Differences were observed among the leachates and exudates, as well as, in the other non humic DOC fractions. However *Chlamydomonas* and *Chlorella* exudates had similar proportions of all fractions and so did red oak and witch hazel leachates. The effect of amendments on bacterial response was determined based on bacterial abundance (DAPI counts, 16S rRNA qPCR), community structure (16S rRNA TRFLP), denitrification potential (chloramphenicol amended acetylene block technique), denitrifier community structure (*nos Z* TRFLP) and abundance (*nos Z* qPCR). Differences were observed in DAPI counts among single treatments in each category as well as between single and mixed treatments, with few exceptions. However, 16S rRNA gene abundance was mostly similar among treatments and did not follow the same trend as DAPI counts. Bacterial community structure also demonstrated differences among treatments with the exception of most aerobic communities. TRFLP peak numbers, however, were not reflective of differences in relative abundance of peaks. Denitrification rates, on average, were three times lower, in mixtures of both chemically defined labile and recalcitrant compounds compared to single treatments, while they were below detection with chemically undefined treatments. No significant differences in denitrification rates were observed among single treatments in each category. Denitrifier abundance as well as percent denitrifiers did not differ among treatments. While community structure was different even among single labile treatments as well as
between single and mixed amendments, no differences were observed among recalcitrant treatments that were chemically defined. Thus, denitrifier structure did not relate to denitrification potential. Although bacterial responses are generally studied and compared among broad DOC categories, this study demonstrates that differences may occur due to molecular heterogeneity even within each category.

INTRODUCTION

Dissolved organic carbon (DOC) is the most abundant form of organic matter in running waters (Karlsson et al., 2005; Tank et al., 2010) and influences bacterial abundance and community composition (Leff and Meyer, 1991; Koetsier et al. 1997; Leff, 2000; Olapade and Leff, 2005). While a large proportion of DOC input in streams is leachate from leaf litter (Kaplan and Newbold, 1993; Giller and Malmqvist, 1998), in-stream primary production can also be an important source of DOC in the form of photosynthetic exudates (Kaplan and Bott, 1989; Gao et al., 2004).

Chemical composition of DOC differs among sources (McDowell and Likens, 1988; Thorp and DeLong, 2002; Tank et al., 2010) and, likewise, the response of bacteria varies based, presumably, on their ability to utilize different compounds (Cottrell and Kirchman, 2000; Olapade and Leff, 2005; Lennon and Cottingham, 2008). For example, Olapade and Leff (2005) treated bacterial communities from stream ecosystems with different DOC sources, i.e., glucose, leaf leachates and algae exudates, and observed that responses of bacterial taxa differed among treatments. While some taxa, like α- and β-
proteobacteria, were more abundant under glucose amendments, gram positive high G+C cells did not show differences in abundance with DOC source. Another study by Cottrell and Kirchman (2000), although performed on marine bacteria, demonstrated differences in utilization of DOC sources by bacterial taxa. In that study, while Cytophaga-Flavobacter dominated uptake of chitin, N-acetylg glucosamine and protein, α-proteobacteria played a major role in the uptake of amino acids. Overall, quality of DOC is an important factor determining bacterial activity and community composition across different ecosystems.

DOC is broadly classified based on molecular weight or bioavailability (Allen, 1976; Jensen, 1983; Hama and Handa, 1987) but even within these broad classes there is considerable heterogeneity in molecular composition (Thurman, 1985; Romani et al., 2006; Vasquez et al., 2011). For example, the labile, low molecular weight compounds include carbohydrates, amino acids and carboxylic acids (Thurman, 1985), which may be utilized differentially by bacterial communities depending on their metabolic capabilities (Cottrell and Kirchman, 2000). The same applies for high molecular weight and recalcitrant DOC compounds (Thurman, 1985).

Previous studies have commonly used generalized descriptors, such as molecular weight or bioavailability, when examining effects of carbon quality on bacterial communities (Leff and Meyer, 1991; Amon and Benner, 1996; Cottrell and Kirchman, 2000) but effect of chemical diversity of DOC has not been examined. For example, labile DOC is more supportive of bacterial growth than recalcitrant compounds.
(Sondergaard and Middleboe, 1995; Weiss and Simon, 1999) but it is not known if bacterial responses differ among different types of labile compounds or if single versus a mixture of labile compounds play a role in such responses.

Stream bacterial communities are diverse in both structure and function (Horn et al., 2011) and it is expected that bacterial responses to DOC properties will vary depending on which attributes of the community are examined (Findlay and Sinsabaugh, 1999). Coarse level measurements, like bacterial abundance or production, are widely studied (Lennon and Cottingham, 2008; William et al., 2010) but do not reveal changes in community composition or in specific biogeochemical processes. Therefore, in this study, bacterial response variables examined included: abundance, community composition and the biogeochemical process of denitrification.

Denitrification is a widely studied process in streams (Mulholland et al., 2008; Groffman et al., 2009; Klocker et al., 2009; Knapp et al., 2009; Kellogg et al., 2010; Mayer et al., 2010) and denitrifiers are able to utilize a diverse set of organic compounds (Zumft, 1997). Denitrification rates are influenced by quality of DOC (Schipper et al., 1994; Hill and Cardaci, 2004) although results from different studies are mixed (Wall et al., 2005; Taylor and Townsend, 2010; Zarnetske et al., 2011). For example, Pfenning and McMohan (1996), in a study of riverine bacteria, demonstrated that acetate and surface-water-derived fulvic acids resulted in higher denitrification rates (estimated from nitrous oxide production) compared to groundwater-derived fulvic acid or sedimentary organic carbon. On the other hand, Wall et al. (2005) demonstrated that sediment
denitrification rates were more dependent on NO$_3$-N concentration rather than DOC. However, such studies have focused on responses of denitrification rates and have typically not examined denitrifier abundance and diversity. Others that have examined structure-function relationships have also demonstrated mixed results. For example, Rich et al. (2003) found a strong correlation between denitrifier community composition and denitrification rates while Boyle et al. (2006) observed no such correlation. It is not known, in streams, if characteristics of the DOC pool impact the rate of denitrification, the denitrifier community or both.

In this study, I investigated the effect of molecular heterogeneity of labile and recalcitrant DOC sources on stream bacterial communities (grown on artificial substrates) using experimental microcosms that were incubated aerobically or anaerobically. Bacterial communities were treated with labile and recalcitrant compounds as single amendments and in mixtures. Bacterial responses measured were bacterial number, bacterial community structure (based on terminal restriction fragment length polymorphisms, or TRFLP, of the 16S rRNA genes, Blackwood et al., 2005), denitrification rates, relative abundance of denitrifiers (based on quantitative PCR of the $nosZ$ gene encoding for nitrous oxide reductase, Henry et al., 2006) and $nosZ$ gene diversity (based on TRFLP of the $nosZ$ gene, Rich et al., 2003). We predicted that different bacterial taxa will respond differentially to the chemical heterogeneity among individual DOC compounds, as well as to the diversity in molecular structure of single versus mixed amendments, resulting in differences in community structure and denitrification potential among treatments.
MATERIALS AND METHODS

Experimental Design and Sample Processing

Laboratory experiments were used to examine bacterial community responses to DOM amendments under aerobic and anaerobic conditions. In both types of experiments, bacterial communities that colonized artificial substrates incubated in the West Branch of Mahoning River in northeastern Ohio (Olapade and Leff, 2005), were used. For aerobic experiments, substrates consisted of unglazed ceramic tiles (25 cm²) that were incubated in coarse mesh bags (0.5cm mesh size) deployed in a riffle in the stream for 5 weeks. For anaerobic experiments, sand (Quikrete Playsand) was placed in nylon cloth bags which were incubated in a deep pool of the stream for 7 weeks.

At the time of substrate deployment and retrieval, in situ temperature, dissolved oxygen concentrations and conductivity were measured in triplicates using HQd/IntelliCAL Rugged Field Kit (Hach Company, Loveland, CO). Water samples from the sites of incubation were also analyzed in triplicates to determine nitrate and soluble reactive phosphorus concentrations, using a LachAT Quikchem 800 FLA+ system (LachAT Instruments, Hach Company, Loveland, CO), and DOC concentrations using Shimadzu TOC analyzer (Shimadzu Corporation, Columbia, MD).

After the stream incubation, initial samples were collected and microcosms were constructed. For aerobic incubations, 7 tiles were added to 2 liter beakers to which artificial stream water (ASW; each liter contained 12 mg NaHCO₃, 7.5 mg CaSO₄ 2H₂O,
7.5 mg MgSO$_4$, 0.5 mg KCl, 10 mg CaCO$_3$, pH 6.4) was also added. Inorganic nutrients were added as K$_2$HPO$_4$ at 10 mg/liter and NaNO$_3$ at 20 mg/L. Water in the beakers was stirred continuously using Phipps and Bird paddle stirrers (Phipps and Bird, Richmond, VA). DOC was added to the ASW to a final concentration of 12 mg C/L for chemically defined compounds and 5 mg C/L for chemically undefined compounds (treatments are in Table 3.1, differences in concentration between the two categories of experiments were based on the concentrations of algal exudates that could be obtained). DOC concentrations were selected based on average concentrations in the study stream (Olapade and Leff, 2005). All treatments were preformed in triplicate and samples were collected after 10 days. Chemical characteristics of water samples from the microcosms, namely pH, DOC and NO$_3^-$-N concentrations, were measured before and after the experiment. pH was measured using a Delta 320 pH meter (Mettler-Toledo, OH). NO$_3^-$-N concentration was measured using LachAT Quikchem 800 FLA+ system (LachAT Instruments, Hach Company, Loveland, CO) and DOC concentrations using a Shimadzu TOC analyzer (Shimadzu Corporation, Columbia, MD).

For anaerobic microcosms, mason jars with septa attached were inoculated with equal volumes of colonized sand and ASW. The head space of the mason jars was evacuated and flushed with helium to create anaerobic conditions. Experimental treatments and conditions were as above for the aerobic microcosms.

Some DOM treatments were undefined chemically and included leaf leachate and algal exudates. Leachates were prepared from red oak (*Quercus rubra*), witch hazel (*Hamamelis virginiana*), corn (*Zea mays*), as well as a mixture of all three, by incubating
in 0.027% NaCl overnight in the dark. Algae exudates were obtained by growing *Chlamydomonas, Chlorella* and *Synedra* cultures (Carolina Biological Supplies, Burlington, NC) in Bristol’s medium (10 ml NaNO₃, 10 ml CaCl₂·2H₂O, 10 ml K₂HPO₄, 10 ml KH₂PO₄, 10 ml MgSO₄·7H₂O, 10 ml NaCl in 940 ml dH₂O). Sodium metasilicate (Na₂SiO₃) was added to facilitate growth of the diatom *Synedra*. The cultures were initially grown for 14 days and further maintained by subculturing every 14 days. Both the leaf leachates and algae exudates were filtered through GF/F filters (Whatman, Maidstone, UK) to remove larger particles and then filter sterilized via 0.02µm Anodisc filters (Whatman, Maidstone, UK) prior to their addition to the microcosms.

DOC from leaf leachates and algae exudates was initially separated into the humic and non-humic fractions. The latter was further divided into basic (includes aromatic amines, protein, amino acids, aminosugars), hydrophobic acid (includes sugar acids, fatty acids, hydroxyl acids) and neutral (includes carbohydrates) fractions as in Wallace et al. (2008). Non-ionic DAX-8 resin (Supelco, Sigma-Aldrich, MO) was used to separate humic from non-humic fractions. While the humic fraction was retained in the DAX-8 resin, the non-humic fraction was obtained as the eluant. Later the humic fraction was recovered from the resin using 5M NaOH. For the non-humics, IR120H cation exchange resin (hydrogen form, Sigma-Aldrich, St. Louis, MO) was used to retain the basic fraction followed by IRA402, the anion exchange resin (chloride form, Fluka, Sigma-Aldrich, St. Louis, MO), used to separate the acidic from the neutral fraction. Thus, the final eluant from IRA 402 comprised of the neutral fraction. DOC concentrations from
each fraction as well as the resin bleed were measured at every step and the concentration of each fraction was calculated as per Wallace et al. (2008).

At the end of the experiments, tiles from the aerobic microcosms were scraped with a known volume of sterile deionized water; a portion was frozen at -80°C for DNA extraction and the remainder was preserved in 4% paraformaldehyde and stored at 4°C for bacterial enumeration. Sand from the anaerobic microcosms was likewise preserved and frozen; additional subsamples were used to measure denitrification as described below.

Samples preserved for bacterial enumeration were treated with 0.1% tetrasodium pyrophosphate and sonicated in a Branson 2210 ultrasonic bath (Branson, Danbury, CT) for 5 minutes. Bacterial abundance was determined after staining with 4, 6- diamidino-2- phenylindole (DAPI) followed by epifluorescence microscopy. A total of fifteen fields were counted for each sample and averaged.

**Denitrification rates**

Sand from the anaerobic microcosms was used to determine denitrification rates via the chloramphenicol amended acetylene block technique as described previously by Royer et al., 2004. A known volume of each sample was placed into two glass bottles fitted with a cap and septum with ASW; chloramphenicol (1 mM initial concentration) was added to one bottle of each pair. Bottles were flushed with helium to create an anoxic environment. 15 ml of purified acetylene was injected into each bottle, and then the bottles were incubated for 4 hours. Headspace samples were collected 15 minutes after
acetylene addition and then after every hour until the end of the incubation. Headspace samples were replaced with a mixture of 90% acetylene and 10% helium. Nitrous oxide concentrations in the samples were measured by automated detection using a Shimadzu GC-2014 Gas Chromatograph (Shimadzu Corporation, Columbia, MD). Denitrification rates were calculated based on changes in nitrous oxide concentration over time and expressed based on dry weight of sand from the respective microcosms.

Denitrification rates were also measured based on nitrous oxide concentration of headspace samples of the microcosms after 72 (3 days), 144 (6 days) and 216 (9 days) hours of incubation. 5ml of gas sample was collected from the headspace of each microcosm and replaced with a similar volume of helium. The nitrous oxide concentration obtained from the headspace samples was measured using the gas chromatograph as above and denitrification rate was calculated in a similar manner.

Based on responses in the experiments described above, an additional experiment was used to look at patterns of change over time in denitrification rates for two treatments (each performed in duplicate): a single compound (glycine) and a mixture of labile compounds (final concentration for both treatments=12mgC/L.) Anaerobic microcosms were set up with sand incubated in the stream for 7 weeks as in the previous experiments. Initial denitrification rates were measured by acetylene block technique as described above. Microcosms containing no amendments served as controls. Denitrification rates of sand samples from these microcosms were measured after 1, 2, 4, 7 and 10 days of incubation using the acetylene block technique as before.
Molecular analyses

DNA of samples from both types of microcosms was extracted using Power soil DNA isolation kits (MoBio, Carlsbad, CA) and quantified by picogreen staining (Sanda et al. 1998) using lambda DNA (Promega) as a standard and Gene 5 software (Biotek). DNA extracts from aerobic microcosms were used for TRFLP analysis and quantitative PCR (qPCR) of the 16S rRNA genes. In addition to 16S TRFLP and qPCR, samples from anaerobic microcosms were subjected to qPCR and TRFLP of the nosZ gene as well.

For TRFLP of 16S rRNA gene, PCR reactions were performed using an equimolar mixture of 5'-ACTCCTACGGGAGGCWGC,3' (Eub338F-0-III) and 5'-ACACCTACGGGTGGCWGC-3' (Eub338F-I-II) for the forward primer and 5'-ACGGGCGGTGTGTACA-3' (1392R) for the reverse primer (W= A or T; Blackwood et al., 2005). A 25μl reaction mixture contained GoTaq Flexi DNA polymerase (2.5 U), buffer (1X), MgCl₂ (0.5 mM), bovine serum albumin (0.64 mg ml⁻¹), deoxynucleoside triphosphates (0.2 mM each), forward and reverse primers (0.2 μM each) along with template DNA. The forward primers were fluorescently labeled with 6-carboxyfluorescein (6-FAM). PCR reactions were carried out in a PTC 200 DNA Engine Cycler (Biorad, Hercules, CA) with the thermal profile being 94°C for 3 min and 30-45 cycles of 94°C (30 s), 57°C (30 s), and 72°C (1 min 30 s) followed by a final extension of 72°C for 7 min. Five reactions were carried out for each sample and products were pooled before performing gel electrophoresis on 1% agarose gels stained with ethidium bromide to check sizes of products. The pooled PCR products were purified using the Qiaquick
PCR purification kit (Qiagen, Valencia, CA) and digested with endonuclease HaeIII (2U) at 37°C for 18 to 24 hours. T-RFLP analysis was performed at The Ohio State Plant Microbe Genomics Facility using a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) and the results analyzed via GeneMapper 4.0 (Applied Biosystems, Foster City, CA).

TRFLP analysis of nosZ gene was done to look at the community fingerprint of denitrifiers in the anaerobic microcosms. Primer sequences used were as described in Rich et al. (2003): 5'-CGCTGTTCITCGACAGYCAG-3' for the forward primer (nosZ-F-1181) and 5'-ATGTGCAKIGCRTGGCAGAA-3' for the reverse primer (nosZ-R-1880) (I= inosine; Y= T and C; K= T and G; R= A and G). 6-Fam (6-carboxyfluorescein) was used to fluorescently label the forward primer. Each 25 µl reaction contained 2µl template DNA, GoTaq Flexi DNA polymerase (2.5 U), ammonium PCR buffer (1x), MgCl₂ (0.5 mM), deoxynucleoside triphosphates (0.8 mM each), and forward and reverse primers (0.8 µM each). The thermal profile for the PCR reaction was as follows: 94°C for 3 min, 50-55 cycles of 94°C (45 s), 55°C (1 min), and 72°C (2 min) followed by a final extension of 72°C for 7 min.

To assess abundance of denitrifiers in anaerobic microcosms, nosZ qPCR was preformed as described by Henry et al. (2006). The forward primer sequence was 5’-WCSYTGTTCMTCGACAGCCGA-3’ (nosZ 1F) and the reverse primer was 5’-ATGTCGATCARCTGVKCRTTYTC-3’ (nosZ 1R) (W = A/T, S = C/G, Y = C/T, M = A/C, R = A/G, K= G/T, and V=A/C/G). Each 25µl of reaction mixture contained
template DNA, SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and primers (0.2 µM each). qPCR reactions were carried out with a Stratagene MX3005P Real-time PCR System (Agilent Technologies, Santa Clara, CA) and thermal cycling conditions comprised of an initial cycle of 95°C for 15 min; 6 cycles of 95°C for 15 s, 67°C for 30 s with a touchdown of -1°C per cycle, 72°C for 30 s, and 80°C for 15 s (data acquisition step); 40 cycles of 95°C for 15 s and 62°C for 15 s, 72°C for 30 s, and 80°C for 15 s; and 1 cycle at 95°C for 15 s and 60°C for 15 s, to 95°C for 15 s.

Approximate abundance of bacteria from the anaerobic and aerobic mesocosms was determined by performing qPCR on 16S rRNA genes. Primer sequences used were as described by Fierer et al. (2005) and consisted of an equimolar mix of 5’-ACTCCTACGGGAGGCAGCAG, 3’, 5’-ACACCTACGGGTGGCTGC, 3’ and 5’-ACACCTACGGGTGGCAGC, 3’ for the forward primer and 5’-ATTACCGCGGCTGCTGG, 3’ for the reverse primer (Eub518R). Other components of the PCR reaction mixtures were the same as for nosZ. The thermal cycling conditions were: 95°C for 10 min then 40 cycles of 94°C (30 s), 57°C (1 min) and 72°C (30 s) (data acquisition step). For qPCR of both nosZ and 16S rRNA genes, a dissociation curve was generated via forty 30 second cycles, increasing 1°C per cycle, starting at 55°C. 

*Pseudomonas aeruginosa* (ATCC number BAA-47; GenBank accession number AE004091) genomic DNA was used as a standard.
Statistical analysis

One-way ANOVA, using JMP statistical software (version 8), was performed to compare the effects of treatment on bacterial abundance, denitrification rates, relative denitrifier and 16S rRNA gene abundance as well as TRFLP peak numbers.

To analyze differences in DOC macrofraction profiles of undefined treatments, namely algae exudates and leaf leachates, one way NPMANOVA followed by principal component analysis (PCA) was performed using the R statistical software (ver. 2.13.2 for Windows).

Redundancy analysis was used to determine the contribution of differences in DOC amendments to the variation among TRFLP profiles (Blackwood et al., 2003). Differences in relative peak heights (Hellinger distance) as well as peak presence or absence (Jaccard distance) were considered to determine differences between profiles and analyses were performed using R statistical software (ver. 2.13.2 for Windows). TRFLP relative peak heights were square root transformed prior to analysis.

RESULTS

Physicochemical variables at study site

Physicochemical variables, namely water temperature, dissolved oxygen,
Table 3.1: DOC amendments used in experimental microcosms. Each of these amendments was administered as single treatments or in mixtures with the other amendments in the same category.

<table>
<thead>
<tr>
<th>Category</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defined labile</td>
<td>Glucose, Glycine, Acetate</td>
</tr>
<tr>
<td>Defined recalcitrant</td>
<td>Coumaric acid, Vanillic acid, Syringic acid</td>
</tr>
<tr>
<td>Undefined labile</td>
<td>Exudates of <em>Chlamydomonas, Chlorella</em> and <em>Synedra</em></td>
</tr>
<tr>
<td>Undefined recalcitrant</td>
<td>Leachates of Red Oak, Witch Hazel and Corn</td>
</tr>
</tbody>
</table>
conductivity, DOC and nutrient concentrations, were measured in triplicates on 20-May, 14-June, 10-July, 17-September and 10-November of 2010 over the period of incubation of the artificial substrates in the stream (Table 3.2). Water temperature changed over the study period and was significantly different among dates (P<0.05, F= 1377.23, df= 4, one way ANOVA). Water temperature was highest on 14- June thereby decreasing in the order of the respective dates in July, September, May and November, 2010 (Tukey HSD).

Dissolved oxygen concentration (Table 3.2) differed significantly among dates (P< 0.05, F= 112.95, df= 4, one way ANOVA). Concentration was highest on 10- November followed by a decrease, in order, from September, May, June and July, 2010 (Tukey HSD).

Conductivity of water samples (Table 3.2) differed among sampling dates (P< 0.05, F= 1948.93, df= 4, one way ANOVA). Highest conductivity was observed on 20- May, 2010 and decreased in the order of 10-November, 10-July, 17-September and 14- June, 2010 (Tukey HSD).

DOC concentration (Table 3.2) differed significantly among the sampled dates (P< 0.05, F= 127.68, df= 4, one way ANOVA). Water samples from May, June and November had higher DOC concentrations compared to those of July and September, 2010 (Tukey HSD). The average DOC concentration was 6.56 mg/L over the incubation period of artificial substrates, which was similar to the concentration of DOC added to the microcosms in the experiment.
Nitrate-N concentrations (Table 3.2) differed between July and the other months (P< 0.05, F= 15.95, df=4, one way ANOVA). Concentration on 10-July was higher than the other dates sampled (Tukey HSD). Average NO$_3^-$-N concentration in the stream was 0.8mg/L and was lower than the initial concentration in artificial stream water (3.3 mg/L NO$_3^-$-N) added to microcosms used for the laboratory experiments.

Soluble reactive phosphorus (SRP) concentrations (Table 3.2) did not differ significantly over time in the study stream (P= 0.08, F= 2.83, df= 4, one way ANOVA). Average SRP concentration over the incubation period of artificial substrates in the stream was 2.6 µg/L, which was lower than the concentration of K$_2$HPO$_4$-PO$_4^{3-}$ (5.45mg/L) added to the artificial stream water used in the microcosms.

**DOC fractions from undefined treatments**

Ion exchange chromatography of leaf leachates and algae exudates (Fig. 3.1) demonstrated significant differences in relative concentrations, of the five DOC fractions measured, among the different sources (NPMANOVA, P< 0.05, F= 30.1, df = 5). The five DOC fractions measured were: basic (BaS), hydrophilic acids (HiA), hydrophobic neutrals (HoN), hydrophilic neutrals (HiN) and aquatic humic substance (AHS). NPMANOVA was followed by a PCA analysis.
Table 3.2: Physicochemical conditions at the study site over the artificial substrate incubation period. Values are means and standard errors obtained from triplicate readings at each time point in the same site.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temp (°C)</th>
<th>DO (mg/L)</th>
<th>Conductivity (µS/cm)</th>
<th>DOC (mgC/L)</th>
<th>SRP (mgP/L)</th>
<th>Nitrate (mgN/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-May</td>
<td>18.63±0.03</td>
<td>8.04±0.06</td>
<td>950.33±2.19</td>
<td>7.92±0.17</td>
<td>0.4±0.03</td>
<td>0.72±0.03</td>
</tr>
<tr>
<td>14-June</td>
<td>22.43±0.19</td>
<td>7.03±0.19</td>
<td>528.33±3.38</td>
<td>7.47±0.12</td>
<td>0.34±0.07</td>
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<td>591.33±0.33</td>
<td>5.21±0.07</td>
<td>0.66±0.07</td>
<td>1.43±0.07</td>
</tr>
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<td>8.86±0.32</td>
<td>572.33±5.13</td>
<td>4.33±0.14</td>
<td>0.48±0.01</td>
<td>0.79±0.03</td>
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<td>10-November</td>
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<td>9.64±0.04</td>
<td>618.00±4.91</td>
<td>7.87±0.26</td>
<td>0.71±0.21</td>
<td>0.88±0.13</td>
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Fig. 3.1: DOC concentration of fractions obtained by ion exchange chromatography of algae exudates and leaf leachates, expressed as a percentage of total DOC obtained from the corresponding source. The fractions are as follows: HiN= hydrophilic neutrals, HiA= hydrophilic acid, BaS= bases, HoN= hydrophobic neutrals, AHS= hydrophobic acids. Algae exudates and leaf leachates were obtained from Chlam= Chlamydomonas; Chlor= Chlorella; Syned= Synedra; RO= red oak; WH= witch hazel and corn.
Results from PCA analysis suggested a difference between algal exudates and leaf leachates with respect to the percentage concentration of all DOC fractions within each category. While certain DOC sources had similar percentage of macrofractions, differences were observed among others. PC axis 1 explained 58% while PC axis 2 explained 19% of the variation. For example, even though Synedra exudate had higher percentage of HiN and HiA fractions compared to Chlorella and Chlamydomonas exudates, the latter were higher in the percentage of HoN and BaS. Chlamydomonas and Chlorella exudates were similar in terms of the percent concentration of DOC fractions. Witch hazel had higher percentage of HoN but lower percentage of BaS fractions compared to red oak and corn. Percent concentration of DOC fractions was similar between red oak and corn leachates.

Aerobic microcosms

Chemically defined treatments

Labile compounds: Bacterial counts, obtained after DAPI staining, were significantly different among amendments, control microcosms and initial samples (P< 0.05, F= 22.22, df = 5, one way ANOVA) in the case of microcosms treated with chemically defined labile DOC sources (Fig. 3.2A). Amendment with the mixture of labile compounds resulted in counts that were significantly higher than those of glucose.
and glycine alone but not significantly different than the acetate amendment (Tukey HSD). For the single DOC treatments, glucose and glycine amended microcosms demonstrated lower counts compared to those treated with acetate (Tukey HSD). DAPI counts of the control microcosms and initial samples were lower than those of the amended microcosms but were not significantly different from each other.

In contrast, differences were observed in bacterial abundance, determined by 16S rRNA gene copy number, between non-amended microcosms and those treated with chemically defined, labile compounds (P<0.05, F= 6.92, df = 5, one way ANOVA) but not among amendments (Fig. 3.3A). For example, microcosms treated with glucose, glycine, acetate and the labile mixture had higher gene copy numbers compared to the initial samples but there were no differences among treatments. Also, bacterial counts from the control, that contained no amendment, were lower than those in the glucose, glycine, acetate and labile mix amended microcosms (Tukey HSD).

Bacterial communities treated with chemically defined labile compounds demonstrated differences in community structure, as determined by redundancy analysis of 16S rRNA gene TRFLP peaks (P< 0.05, F= 3.69, df = 5, Fig. 3.4A). 36% of the variation in community structure was explained by amendment. The labile DOC amended microcosms were not only different from each other, with the exception of glucose and glycine amended communities, but also from the initial community. Furthermore, the bacterial community structure of the control microcosms was different from those of the initial and single labile DOC amended communities.
Fig. 3.2: Bacterial abundance from DAPI counts of aerobic microcosms amended with chemically defined A) labile and B) recalcitrant DOC compounds. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to counts prior to amendments. CA = coumaric acid, SA = syringic acid and VA = vanillic acid. Values are means and standard errors.
Fig. 3.3: 16S rRNA gene abundance of bacteria incubated in aerobic microcosms amended with chemically defined A) labile and B) recalcitrant DOC compounds. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to abundance prior to amendments. CA = coumaric acid, SA = syringic acid and VA = vanillic acid. Values are means and standard errors.
Fig. 3.4: RDA axes plots obtained from redundancy analysis of 16S rRNA gene TRFLP peak relative abundance, in aerobic microcosms amended with chemically defined A) labile and B) recalcitrant DOC compounds. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to community structure prior to amendments. CA= coumaric acid, SA= syringic acid and VA= vanillic acid.
Number of peaks (from 16S rRNA gene TRFLP analysis) of the initial community as well as that of a chemically defined labile amendment (Fig. 3.5A), namely acetate, was significantly higher than the control (P< 0.05, F= 4.41, df = 5, one way ANOVA followed by Tukey HSD). However, no significant differences in peak numbers were observed among the single labile treatments or between the single and mixed treatments.

Differences in initial and post-experimental DOC concentrations of the water column (Fig. 3.6A) was observed (P< 0.05, F= 20.88, df = 7, one way ANOVA); final DOC concentrations at the end of the experiment was significantly lower than initial concentrations (Tukey HSD). There was no significant difference in final DOC concentrations among treatments after the experiment. DOC concentration in the control microcosms were below the detection limit.

pH of the water column measured before and after the experiment (Fig. 3.7A) was significantly different only in a few microcosms treated with chemically defined labile compounds (P< 0.05, F= 13.08, df = 9, one way ANOVA). For example, pH of microcosms treated with glucose and mixture of labile compounds demonstrated a significant increase at the end of the experiment while the other microcosms demonstrated no significant change. Also, there was no significant difference in pH among microcosms at the end of incubation.
Fig. 3.5: Number of peaks obtained from 16S rRNA gene TRFLP profiles from aerobic microcosms amended with chemically defined A) labile and B) recalcitrant DOC compounds. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to peak numbers prior to amendments. CA = coumaric acid, SA = syringic acid and VA = vanillic acid. Values are means and standard errors.
Fig. 3.6: Dissolved organic carbon concentration of the water column in aerobic microcosms before (initial) and after (final) the experiment for chemically defined A) labile, B) recalcitrant treatments. CA= coumaric acid, SA= syringic acid and VA= vanillic acid. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments.
Fig. 3.7: pH of the water column in aerobic microcosms before (initial) and after (final) the experiment for chemically defined A) labile, B) recalcitrant treatments. CA= coumaric acid, SA= syringic acid and VA= vanillic acid. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments.
Recalcitrant compounds: DAPI counts from the recalcitrant DOC treated microcosms (Fig 3.2B) demonstrated significant differences among amendments, control microcosms and initial samples (P< 0.05, F= 18.70, df = 5, one way ANOVA). The syringic acid amendment resulted in counts that were significantly higher than those of coumaric acid but not so compared to the other treatments (Tukey HSD). Counts from coumaric acid amended microcosms were also lower than those of the recalcitrant mixture but similar to that of the control microcosms. Amendments, other than coumaric acid, demonstrated counts that were higher than those of the control microcosms. Comparison of bacterial counts before and after the experiment showed lower counts in the control as well as coumaric acid amended microcosms than the initial samples. However, no significant difference was observed between the initial counts and those from the other microcosms.

16S rRNA qPCR copy numbers for the recalcitrant treatments (Fig. 3.3B) demonstrated significant differences among amendments, controls, and initial samples (P< 0.05, F= 5.6, df = 5, one way ANOVA). Amendment with vanillic acid showed higher copy numbers compared to coumaric acid, syringic acid and control microcosms as well as the initial samples (Tukey HSD). No significant differences were observed among the single and mixed recalcitrant treatments, unlike those observed for DAPI counts; or among the control, initial samples and amendments other than vanillic acid.

On the other hand, differences in bacterial community structure (from redundancy analysis of 16S rRNA gene TRFLP peaks) were not explained significantly by treatment
Although, based on RDA axes, it was observed that the initial communities and those treated with the mixture of recalcitrant compounds were spatially separated from the individual DOC amended communities and control; treatment explained only 14% of the variation. Even though DAPI and 16S rRNA gene abundance were different among some treatments, bacterial community structure remained similar.

Number of peaks obtained from 16S rRNA gene TRFLP analysis (Fig. 3.5B) were different among treatments as well as with the control and initial samples (P< 0.05, F = 9.16, df = 5, one way ANOVA). Peak numbers from bacterial samples treated with coumaric acid were significantly higher those from syringic and vanillic acid treatments as well as from the control and initial samples (Tukey HSD). These differences were observed in spite of a clustering of the DOC amended communities on the RDA axes after redundancy analysis. No difference was, however, observed in peak number between the single and mixed recalcitrant treatments.

DOC concentration of the water column in microcosms treated with recalcitrant compounds (Fig. 3.6B) were different after the experiment and also among amendments at the end of the incubation period (P<0.05, F = 82.73, df = 7, one way ANOVA). DOC concentrations in the water column at the end of the experiment were significantly lower than initial concentrations (Tukey HSD). Microcosms amended with syringic acid showed higher final DOC concentration compared to that of vanillic acid, after the experiment. DOC concentration in the control microcosms were below detection limit.
Microcosms treated with recalcitrant compounds did not demonstrate a significant difference in pH before and after the experiment or among microcosms at the end of the experiment (Fig. 3.7B). The only differences observed were between the final and initial pH across microcosms (P< 0.05, F= 3.19, df = 9, one way ANOVA). For example, although no difference was observed between the initial and final pH of recalcitrant mixture and control microcosms considered separately, pH of the control microcosms at the end of the experiment was higher than that of the initial pH of the microcosm treated with the mixture of recalcitrant compounds (Tukey HSD).

Chemically undefined treatments

*Algal Exudates:* DAPI counts from microcosms treated with algal exudates (Fig. 3.8A) did not demonstrate a significant difference among amendments, controls or initial samples (P= 0.13, F= 2.30, df = 5, one way ANOVA). However, abundance based on 16S rRNA copy numbers (Fig. 3.9A) did demonstrate differences among amendments, control microcosms and initial samples (P< 0.05, F= 29.9, df = 5, one way ANOVA). Bacteria from control microcosms demonstrated significantly lower gene copy number compared to the treatments whereas the initial number of copies were higher compared to those after amendment. Although DAPI counts were similar among all treatments, 16S rRNA gene copies were significantly higher in microcosms treated with *Synedra* exudates compared to those treated with *Chlamydomonas* exudates (Tukey HSD). No significant differences were observed among the other treatments.
Fig. 3.8: Bacterial abundance from DAPI counts of aerobic microcosms amended with A) algal exudates and B) leaf leachates. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to counts prior to amendments. Chlam= *Chlamydomonas*, Chlor= *Chlorella*, Syned= *Synedra*. Values are means and standard errors.
Fig. 3.9: 16S rRNA gene abundance of bacteria incubated in aerobic microcosms amended with A) algal exudates and B) leaf leachates. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to gene abundance prior to amendments. Chlam= *Chlamydomonas*, Chlor= *Chlorella*, Syned= *Synedra*. Values are means and standard errors.
In terms of community structure, based on relative abundance of 16S rRNA gene TRFLP peaks, bacteria treated with algal exudates (Fig. 3.10A) were different from the initial communities (P<0.05, F=2.04, df=5) but amendment explained only 3% of the variation. The exudate amended communities clustered together on the RDA axes. The difference between *Chlorella* and *Synedra* exudates in terms of 16S rRNA gene copy number was not reflected in community structure. However, differences observed between experimental and the initial community structure were also seen in 16S rRNA gene copy numbers. Lack of significant difference among treatments observed with DAPI counts were in agreement with lack of difference in community structure.

No difference in 16S rRNA TRFLP peak numbers (Fig. 3.11A) was observed among any of the communities, including control and initial communities, with algal exudates treatment (P=0.78, F=0.48, df=5, one way ANOVA). This lack of difference among exudate amended communities was reflective of the clustering of those on the RDA axes obtained from the redundancy analysis of their relative abundance. However, spatial segregation of the initial from exudate amended communities on the RDA axes was not reflected in terms of difference between them in the number of TRFLP peaks.

Initial DOC concentration of artificial stream water in aerobic microcosms amended with algal exudates (Fig. 3.12A) was significantly different from that at the end of the incubation period (P<0.05, F=16.25, df=7, one way ANOVA). DOC concentration after the experiment demonstrated a significant decrease from the initial concentration of 5mg/L (Tukey HSD). However, no differences were observed in DOC
concentrations among the exudate amended microcosms at the end of the experiment. In this case, too, DOC concentration in the control microcosms was below detection limit.

Treatment with algal exudate did not result in a significant change in pH (Fig. 3.13A) before and after the incubation period and the only difference observed was with the initial pH of some microcosms with the post-experimental pH of others (P< 0.05, F= 3.47, df = 9, one way ANOVA). For example, pH of microcosms treated with Synedra exudates, at the end of the experiment, was significantly higher than the initial pH of those treated with the mixture of exudates and the control microcosm (Tukey HSD). Also, no difference in pH was observed among microcosms after the experiment.

Leaf leachate: Amendment with leaf leachates led to significant differences in DAPI counts (Fig. 3.8B) among treatments, control, and initial samples (P< 0.05, F= 13.45, df = 5). Control microcosms had lower bacterial numbers compared to corn and red oak leachate as well as initial samples (Tukey HSD). These latter also demonstrated higher bacterial counts compared to microcosms amended with witch hazel leachate. Furthermore, corn leachate led to higher bacterial abundance compared to the mixture of leachates. Initial bacterial abundance was similar to that of the final abundance in the amendments; witch hazel was an exception to this trend for which bacterial counts were lower at the end of the experiment than they were initially.
Fig. 3.10: RDA axes plots obtained from redundancy analysis of 16S rRNA gene TRFLP peak relative abundance, in aerobic microcosms amended with A) algal exudates and B) leaf leachates. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to communities prior to amendments. Chlam = Chlamydomonas, Chlor = Chlorella, Syned = Synedra. Values are means and standard errors.
Fig. 3.11: Number of peaks obtained from 16S rRNA gene TRFLP profiles of bacterial communities incubated in aerobic microcosms with A) algae exudates and B) leaf leachates. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to peak numbers prior to amendments. Chlam= Chlamydomonas, Chlor= Chlorella, Syned= Synedra. Values are means and standard errors.
Fig. 3.12: Dissolved organic carbon (DOC) concentration of the water column in aerobic microcosms before (initial) and after (final) the experiment for amendments with A) algae exudates and B) leaf leachates. Chlam = Chlamydomonas, Chlor = Chlorella, Syned = Synedra. Mix indicates a mixture of compounds in the same category. Values are means and standard errors.
Fig. 3.13: pH of the water column in aerobic microcosms before (initial) and after (final) the experiment for amendments with A) algae exudates and B) leaf leachates. Chlam= *Chlamydomonas*, Chlor= *Chlorella*, Syned= *Synedra*. Mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments. Values are means and standard errors.
Table 3.3: Summary of results obtained from aerobic microcosms amended with chemically defined and undefined labile and recalcitrant compounds, as individual treatments and their mixtures, and incubated over a period of 10 days. The results are based on statistical analysis of data obtained from the microcosms using one way ANOVA followed by post hoc Tukey for all except bacterial community structure in which case redundancy analysis of 16S r RNA gene TRFLP peaks was performed. Only significant differences (P< 0.05) among values have been reported. Glu= glucose, Gly= glycine, Ac = Acetate; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; Chlam= Chlamydomonas; Chlor= Chlorella; Syned= Synedra; RO= red oak; WH= witch hazel, S= significant difference, NS= no significant difference.
<table>
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<th>16S rRNA gene abundance</th>
<th>Community structure</th>
<th>16S rRNA TRFLP peak numbers</th>
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<td></td>
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<td>NS</td>
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<td>NS</td>
<td>Corn final&gt; initial, among treatments NS</td>
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<tr>
<td></td>
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<td>NS</td>
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Differences were observed among treatments, control and initial samples in terms of 16S rRNA gene copy numbers (P< 0.05, F= 58.69, df = 5, one way ANOVA, Fig. 3.9B) but unlike DAPI counts no significant differences were observed among amendments (Tukey HSD). As was observed for DAPI counts, the control microcosms had the lowest gene copy number, and the initial samples had higher gene copy numbers compared to all the other treatments.

Amendment with leaf leachate resulted in differences between initial and post-experimental community structure, as observed from differences in relative abundance of 16S rRNA gene TRFLP peaks (P< 0.05, F= 2.04, df = 5, one way ANOVA, Fig. 3.10B). Treatment explained 5% of the variation. However, in this case too, as in algae exudate treatments, the leachate amended communities grouped together on the RDA plot axes. Communities from the control microcosms also clustered together with the leachate-amended communities but away from the initial communities. Differences in 16S rRNA gene copy numbers, as well as DAPI counts, between initial samples and the microcosms was in agreement with such differences in community structure. However, differences in gene copy number between the control microcosms and those amended with leachate did not translate to differences in community structure. Likewise, DAPI counts were also different among treatments even though the community structure was similar.

The pattern observed from redundancy analysis of 16S rRNA TRFLP relative peak abundance was repeated in terms of the total peak number (Fig. 3.11B). There was a significant difference between control as well as red oak leachate amended communities
with initial community peak numbers (P< 0.05, F= 4.03, df = 5, one way ANOVA), the initial communities having more number of peaks (Tukey HSD). Once again, as observed in case of community structure, no difference in the number of peaks was observed among the leachate amended communities or between those and the control.

Leaf leachate amended microcosms did not demonstrate a significant change in DOC concentration (Fig. 3.12B) at the end of the experiment when compared to the initial concentration of 5 mg/L (P= 0.05, F= 3.25, df =7, one way ANOVA followed by Tukey HSD). A similar lack of difference was also observed when DOC concentrations were compared among microcosms at the end of the experiment. As was the case previously, DOC concentration in the control microcosms was below detection limit.

A significant difference was observed between the initial and post-experimental pH (Fig. 3.13B) of microcosms amended with corn leachate (P< 0.05, F= 8.03, df = 9, one way ANOVA). pH increased at the end of the experiment from the initial values in these microcosms (Tukey HSD). This change was, however, not observed in the other microcosms and final pH values of all experimental microcosms were similar.

Anaerobic microcosms

Unlike the aerobic microcosms, anaerobic microcosms were set up from the same initial samples and had the same controls for both chemically defined labile as well as
recalcitrant treatments. This applied for algae exudates and leaf leachate treatments as well, where the same initial communities were exposed to the two DOC categories, and were run concurrently with the same control microcosms.

Chemically defined treatments

Labile Compounds: In anaerobic microcosms, treated with chemically defined labile compounds, DAPI counts (Fig. 3.14) differed significantly between initial and post-experimental samples but not among treatments (P< 0.05, F= 6.84, df = 5, one way ANOVA). Counts were significantly lower in the initial samples compared to all except the controls (Tukey HSD), with which it demonstrated no significant difference.

On the other hand, 16S rRNA gene copies (Fig. 3.15) was significantly different among treatments, control microcosms and initial samples (P< 0.05, F= 34.41, df = 5, one way ANOVA) for these microcosms. While the control microcosm and initial samples had lower copy numbers compared to all the DOC amended microcosms, differences were also observed in 16S rRNA gene copy number from microcosms amended with acetate and the mixture of labile compounds, the latter being higher (Tukey HSD). No significant difference in gene copy numbers was observed among single compound treatments. DAPI counts demonstrated a similar trend for control microcosms and initial samples, but the counts were similar among all DOC amendments, unlike the 16S rRNA gene abundance.
Fig. 3.14: Bacterial abundance from DAPI counts of anaerobic microcosms amended with chemically defined labile and recalcitrant DOC compounds. Glu= glucose, Gly= glycine, Ac = Acetate; LM= mixture of labile compounds; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; RM= mixture of recalcitrant compounds; RO= red oak; WH= witch hazel; LfM = mixture of leachates; Chlam= Chlamydomonas; Chlor= Chlorella; Syned= Synedra; AM= mixture of algal exudates; Ctrl= control; Ini= initial counts. Values are means and standard errors.
Fig. 3.15: 16S rRNA gene abundance of bacteria incubated in anaerobic microcosms amended with chemically defined labile and recalcitrant DOC compounds. Glu= glucose, Gly= glycine, Ac = Acetate; LM= mixture of labile compounds; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; RM= mixture of recalcitrant compounds; Ctrl= control (no amendment); Ini= initial gene abundance. Values are means and standard errors.
Bacterial community structure (Fig. 3.16) differed significantly among amendments (P< 0.05, F= 1.64, df = 5, one way ANOVA), as indicated by difference in relative abundance of 16S rRNA TRFLP peaks, and amendment explained 49% of the variation. Bacterial communities receiving glucose and acetate amendments were different from those treated with glycine, but were similar to each other. Treatment with the mixture of labile compounds resulted in communities similar to glycine treatments and different from glucose and acetate treatments. Bacterial communities from the control microcosm were similar to that of the initial samples and were different from the DOC amended communities.

Number of TRFLP peaks (Fig. 3.17) were however not significantly different among treatments, control microcosms or initial samples (P= 0.37, F= 1.21, df = 5, one way ANOVA) unlike differences observed in relative abundance of 16S rRNA TRFLP peaks among these microcosms.

Denitrification rates (measured as rates of N₂O production) were significantly different between the single and mixed labile DOC treatments (P< 0.05, F= 42.68, df = 5, one way ANOVA) when measured at the end of incubation (Fig. 3.18A). Microcosms treated with glucose and glycine, demonstrated higher denitrification rates compared to those treated with the mixture of these compounds (Tukey HSD). There was, however, no significant difference among the single compound DOC treatments. All microcosms demonstrated denitrification rates higher than the control and initial samples.
Fig. 3.16: RDA axes plots obtained from redundancy analysis of 16S rRNA gene TRFLP peak relative abundance in anaerobic microcosms amended with chemically defined A) labile and B) recalcitrant DOC compounds. CA = coumaric acid; SA = syringic acid; VA = vanillic acid; mix indicates a mixture of the individual compounds in the same category; control indicates microcosms with no DOC amendments and initial refers to the community prior to amendments. Values are means and standard errors.
Fig. 3.17: Number of peaks obtained from 16S rRNA gene TRFLP profiles of bacterial communities incubated in anaerobic microcosms amended with chemically defined labile and recalcitrant DOC compounds. Glu= glucose, Gly= glycine, Ac = Acetate; LM= mixture of labile compounds; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; RM= mixture of recalcitrant compounds; Ctrl= control (no amendment); Ini= initial communities. Values are means and standard errors.
Fig. 3.18: Denitrification rates of anaerobic microcosms based on N$_2$O-N production calculated from A) artificial substrate after laboratory incubation of 10 days B) slope of headspace samples taken after 72, 144 and 216 hours of incubation with chemically defined labile and recalcitrant DOC sources. Glu= glucose, Gly= glycine, Ac = Acetate; LM= mixture of labile compounds; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; RM= mixture of recalcitrant compounds; Ctrl= control (no amendment), Ini= initial rates. Values are means and standard errors.
N₂O-N production rates were based on the slope obtained from the headspace samples of anaerobic microcosms were measured after 72, 144 and 216 hours of incubation (Fig. 3.18B). Chemically defined labile compounds demonstrated differences in rates of denitrification (P< 0.05, F=52.8, df = 4, one way ANOVA). For the labile DOC treatments, glycine had the highest denitrification rates while the control microcosm the lowest (Tukey HSD). Although denitrification from glucose and acetate treatments was similar, and lower than that of glycine, all these microcosms had rates higher than that of the microcosm amended with the mixture of the labile compounds. Treatment with the mixture of glucose, glycine and acetate led to similar denitrification rates as that of the control.

The trends observed in terms of N₂O-N production rates, on comparison of single and mixed treatments with chemically defined labile compounds, from the headspace samples of the microcosms during incubation, were similar to those observed when measurements were made from the substrates using the acetylene block method at the end of the 10 day incubation period. The exception to this trend was that differences were observed in denitrification rates among microcosms amended with single labile compounds, when measured from headspace samples, but not from the artificial substrates. The difference in N₂O-N concentration between the single and mixed treatments was found in both cases, with the single treatments showing higher denitrification rates, regardless of the source, compared to the mixed treatments. Overall, the rates measured from the headspace samples over the incubation period were two
orders of magnitude lower than the ones from the artificial substrates at the end of the experiment.

In addition to headspace sampling, to monitor the trends of change in denitrification rates of single compound treatments and their mixtures, rates were measured intermittently by setting up similar experiments as before using glycine as the single compound treatment and the mixture of glucose, glycine and sodium acetate as the mixed labile treatment (Fig. 3.19). Initial denitrification rate was low and not significantly different from that of the previous experiment. Denitrification rates of both glycine and mixed labile DOC treated microcosms were below detection on days 1 and 2. On day 4, denitrification rate was detectable for the labile mixture but barely above detection for glycine-amended microcosms. On day 7, while glycine treated microcosms demonstrated rates that were below detection limits, mixed labile treatment led to detectable denitrification rates. On day 10, denitrification rates were detectable for both the treatments and similar to the previous experiment higher rates were observed in the single compound treatments than in the mixture. However, rates from both types of microcosms were still an order of magnitude lower than that of the corresponding treatments in the previous experiment with chemically defined labile amendments.

nosZ gene abundance (Fig. 3.20A) was not significantly different among chemically defined labile DOC treated microcosms (P = 0.12, F = 2.32, df = 5, one way ANOVA). No significant differences were observed among DOC treated and initial samples as well as control microcosms.
Relative denitrifier abundance, or nosZ as a percent of 16S rRNA gene copies (Fig. 3.20B), did differ between the chemically defined labile treatments and the initial samples (P< 0.05, F= 6.75, df = 5, one way ANOVA), with the initial samples having lower percentage of denitrifiers compared to the acetate treatment (Tukey HSD). The initial samples and control microcosms had similar relative abundance. There was no significant difference in relative abundance among treatments, however. Control microcosms were similar in relative abundance to both the initial samples and the DOC amended microcosms.

The community fingerprint of denitrifiers obtained from TRFLP analysis of the nosZ gene revealed a difference in structure among microcosms treated with chemically defined labile compounds (P< 0.05, F= 2.02, df = 5; Fig. 3.21A) and 30% of the variation was explained by amendment. This was based on differences in the relative abundance of nosZ gene TRFLP peaks. Although bacterial communities amended with glycine, acetate and glucose are spatially separated on the RDA axes, a clustering of those treated with glucose and the mixture of labile compounds was observed. Such a trend was not seen in denitrification rates which were similar among all single amendments, being higher than the mixture. The community structure of control and initial samples were similar to each other but different from the other microcosms.
Fig. 3.19: Rates of denitrification measured from bacteria grown in anaerobic microcosms incubated with glycine and a mixture of labile compounds after 1, 2, 4, 7 and 10 days. The labile mix comprises of glucose, glycine and sodium acetate.
Fig 3.20: A) nosZ gene abundance and B) relative nos Z gene abundance expressed as a percentage of 16S rRNA gene, in anerobic microcosms incubated with chemically defined labile and recalcitrant DOC sources. Glu= glucose, Gly= glycine, Ac = Acetate; LM= mixture of labile compounds; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; RM= mixture of recalcitrant compounds; Ctrl= control (no amendment), Ini= initial abundance. Values are means and standard errors.
Fig. 3.21: RDA axes plots from redundancy analysis of *nosZ* gene TRFLP peak relative abundance, in anaerobic microcosms amended with chemically defined A) labile and B) recalcitrant DOC compounds. Labile mix indicates a mixture of labile compounds while RM is the mixture of recalcitrant compounds; control indicates microcosms with no DOC amendments and initial refers to community structure prior to amendments. CA= coumaric acid, SA= syringic acid and VA= vanillic acid.
Anaerobic microcosms treated with chemically defined labile compounds demonstrated differences in the number of \textit{nosZ} gene peaks (Fig. 3.22) among treatments as well as with initial and control samples ($P<0.05$, $F=18.39$, $df=5$, one way ANOVA). Denitrifiers treated with acetate demonstrated peak numbers that were similar to those of the control and initial communities but significantly higher than those of glucose, glycine and the labile mixture (Tukey HSD). Although the similarity of controls and initials with acetate amended communities was not observed with redundancy analysis of their relative abundance, the other differences were in agreement.

Nitrate concentration in the water column of these anaerobic microcosms (Fig. 3.23) was measured before and after the experiment. Microcosms demonstrated significant differences between initial and post-experimental nitrate concentrations ($P<0.05$, $F=20.24$, $df=9$, one way ANOVA). There was a significant decrease in nitrate concentration at the end of the experiment for all treatments. There was, however, no significant difference in nitrate concentration among microcosms, including the control, after the 10 day incubation period (Tukey HSD).

A significant decrease over the ten day incubation in DOC concentration (Fig. 3.24) from initial was observed in anaerobic microcosms treated with chemically defined labile compounds ($P<0.05$, $F=25.9$, $df=7$, one way ANOVA). Final DOC concentration, when compared among treatments, was not significantly different (Tukey HSD). DOC concentration in the control microcosms was below detection limits.
Differences were observed in initial and post-experimental pH of the water column (Fig. 3.25) in microcosms amended with chemically defined labile compounds (P< 0.05, F= 59.66, df = 9, one way ANOVA). pH at the end was significantly higher compared to the initial pH at the time incubation started (Tukey HSD). However, the final pH of the microcosms still ranged between 6 and 8 at the end of incubation. There was, however, no difference observed among microcosms in terms of post-experimental pH.

Recalcitrant compounds: The chemically defined recalcitrant treatments exhibited a significant difference in DAPI counts (Fig. 3.14), not only with the initial samples and unamended control, but also with respect to each other (P< 0.05, F= 13.15, df = 5, one way ANOVA). The microcosms treated with syringic acid and vanillic acid demonstrated the highest counts followed by the ones treated with coumaric acid and the mixture of recalcitrant compounds (Tukey HSD). The control microcosm and initial samples were not significantly different from each other and DAPI counts from these samples were significantly lower than syringic acid and vanillic acid treated microcosms.

No significant differences were observed in 16S rRNA gene abundance (Fig. 3.15) among treatments, control and initial samples for microcosms treated with chemically defined recalcitrant compounds (P= 0.06, F= 2.98, df = 5, one way ANOVA). This was unlike the trend observed for DAPI counts.
Fig. 3.22: Number of peaks from nosZ gene TRFLP profiles of anaerobic microcosms amended with chemically defined labile and recalcitrant DOC compounds. Glu = glucose, Gly = glycine, Ac = Acetate; LM = mixture of labile compounds; CA = coumaric acid; SA = syringic acid; VA = vanillic acid; RM = mixture of recalcitrant compounds; Ctrl = control (no amendment), Ini = initial peak numbers. Values are means and standard errors.
Fig. 3.23: NO$_3$-N concentration of the water column in anaerobic microcosms before (initial) and after (final) the experiment for chemically defined labile and recalcitrant treatments. Glu= glucose, Gly= glycine, Ac = Acetate; LM= mixture of labile compounds; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; RM= mixture of recalcitrant compounds. Values are means and standard errors.
Fig. 3.24: Dissolved organic carbon (DOC) concentration of the water column in anaerobic microcosms before (initial) and after (final) the experiment for chemically defined labile and recalcitrant treatments. Glu = glucose, Gly = glycine, Ac = Acetate; LM = mixture of labile compounds; CA = coumaric acid; SA = syringic acid; VA = vanillic acid; RM = mixture of recalcitrant compounds. Values are means and standard errors.
Fig. 3.25: pH of the water column in anaerobic microcosms before (initial) and after (final) the experiment for chemically defined labile and recalcitrant treatments. Glu= glucose, Gly= glycine, Ac = Acetate; LM= mixture of labile compounds; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; RM= mixture of recalcitrant compounds. Values are means and standard errors.
Differences in relative abundance of 16S rRNA TRFLP peaks (Fig. 3.16) suggested a difference in bacterial community structure among bacteria treated with recalcitrant compounds, initial communities and control microcosms ($P<0.05$, $F=2.66$, $df=5$). Treatment explained 52% of the variation. The communities treated with vanillic acid, coumaric acid and the mixture of recalcitrant compounds were similar to each other and different from the ones treated with syringic acid. Bacterial communities from the control microcosms were similar to those of the initial samples, and differed from all other recalcitrant DOC amended communities.

Number of TRFLP peaks (Fig. 3.17) demonstrated a significant difference among control, initial and DOC amendments as well ($P<0.05$, $F=23.03$, $df=5$, one way ANOVA). Initial samples, control microcosms and bacteria from the mixture of recalcitrant treatments demonstrated similar number of peaks, which were higher than peak numbers from bacterial communities treated with coumaric, vanillic and syringic acid treatments (Tukey HSD). The latter three in turn demonstrated no significant difference among themselves in the number of peaks.

As was seen in the case of labile DOC amendments, a similar pattern in denitrification rates, measured from artificial substrates at the end of incubation (Fig. 3.18A), was observed in microcosms amended with recalcitrant DOC ($P<0.05$, $F=8.26$, $df=5$, one way ANOVA). While there was no difference in rates among individual compounds (coumaric, vanillic and syringic acid), single DOC treatments had higher denitrification rates compared to the microcosms treated with a mixture of these
compounds (Tukey HSD). Denitrification rates of microcosms amended with recalcitrant DOC sources were not significantly different from the control and initial samples.

The above trend in denitrification rates, observed at the end of the incubation period, was also seen with respect to rates measured from the periodic headspace samples (Fig. 3.18B) of the microcosms (P< 0.05, F= 17.73, df = 4, one way ANOVA). Treatment with individual recalcitrant compounds demonstrated similar rates; while the microcosm treated with the mixture of recalcitrant compounds had rates that were lower than those treated with coumaric and vanillic acid (Tukey HSD). Denitrification from the mixed treatment was similar to that of the control.

Chemically defined recalcitrant DOC treatments demonstrated significant differences from the control in terms of nosZ gene abundance (P< 0.05, F= 6.23, df = 5, Fig. 3.20A, one way ANOVA). The control microcosm had lower gene abundance compared to the DOC treated microcosms. The only exception to this was the lack of difference between control and coumaric acid treated microcosms (Tukey HSD). Initial gene abundance was not significantly different from final abundance in microcosms, but was higher than the control. Also, nosZ gene abundance was similar among the experimental treatments. Relative denitrifier abundance, or percent nosZ abundance (Fig. 3.20B), was not significantly different among chemically defined recalcitrant treatments, control microcosms as well as the initial samples (P= 0.05, F= 3.45, df = 5).

Differences in community structure of denitrifiers in microcosms treated with recalcitrant compounds (Fig. 3.21B), measured as relative abundance of nosZ gene
TRFLP peaks, was not explained significantly by amendment (P= 0.16, F= 1.18, df = 5). Although communities appeared spatially separated on the RDA axes, treatment explained 19% of the variation. These patterns did not resemble denitrification rates that were higher in single treatments compared to the mixed recalcitrant DOC amendments.

The similarity in community structure of denitrifiers was in agreement with data on the nosZ TRFLP peak numbers (Fig. 3.22) for the recalcitrant treatments. Although a significant difference was observed between the peak number of control and vanillic acid amended communities (P< 0.05, F= 3.4, df = 5, one way ANOVA), control microcosms showing higher numbers than vanillic acid treatments (Tukey HSD), no such difference was observed with the other DOC treated communities or among treatments as well as between initial and post-incubation peak numbers.

A significant difference was found between the initial and post-experimental nitrate concentrations (P< 0.05, F= 36.15, df = 9, Fig. 3.23, one way ANOVA) of microcosms amended with chemically defined recalcitrant DOC compounds. Nitrate concentration of the experimental and control microcosms were lower at the end of the incubation period compared to the initial concentration. There was no difference in nitrate concentration among microcosms after the experiment (Tukey HSD).

A significant decrease in DOC concentration of the water column (Fig. 3.24), from initial values, was observed with recalcitrant treatments (P< 0.05, F= 18.83, df = 7, one way ANOVA followed by Tukey HSD) after the experiment. Comparison of post-
experimental DOC concentration, however, did not show significant differences among treatments. DOC concentration in the control microcosms was below detection limit.

Differences were observed in initial and post-experimental pH (Fig. 3.25) of the water column in microcosms amended with recalcitrant DOC (P< 0.05, F= 26.47, df = 9, one way ANOVA). pH at the end of the experiment was significantly higher compared to the initial pH at the time of incubation, even though the final pH ranged between 6 and 7 (Tukey HSD). There was, however, no difference observed among microcosms in terms of post- incubational pH.

Chemically undefined

*Algal exudates*: DAPI counts (Fig. 3.26) demonstrated a significant difference between experimental microcosms and the control as well as initial samples (P< 0.05, F= 10.11, df = 5, one way ANOVA). The initial samples had counts that were similar to the *Chlorella* exudate treated and control microcosms but lower than the others. The microcosm amended with *Chlorella* exudates, however, did not demonstrate a significant difference from the control or any other microcosm. No significant difference was observed in DAPI counts among other experimental microcosms (Tukey HSD).

The above microcosms demonstrated differences in 16S rRNA gene abundance (Fig. 3.27), among treatments, control microcosms and initial samples (P< 0.05, F= 24.37, df = 5, one way ANOVA). Microcosms treated with *Chlamydomonas* exudates
had higher gene copies compared to those amended with *Synedra* exudates (Tukey HSD). There was no significant difference between the single and mixed exudates treatments. All treatments had higher 16S rRNA gene copy numbers compared to the initial samples and the control. The differences observed among experimental microcosms in 16S rRNA gene abundance, were not seen in DAPI counts.

Treatment with algae exudate resulted in significant differences in the relative abundance of 16S rRNA gene TRFLP peaks, or bacterial community structure ($P<0.05$, $F=7.18$, df = 5, Fig. 3.28A) among amendments as well as with the control microcosms and initial samples. 48.34% of the variation was explained by treatment. Treatment with *Chlorella* exudate led to differences with the *Synedra* and *Chlamydomonas* exudate treated communities. Amendment with mixed exudate resulted in communities similar to *Chlorella* exudate treatment, but different from communities treated with *Synedra* and *Chlamydomonas* exudates. Control microcosms and initial samples had similar community structure that differed from the exudate amended communities.

Number of TRFLP peaks (Fig. 3.29) differed significantly among treatments, control and initial samples ($P<0.05$, $F=40.96$, df = 5, one way ANOVA). The control microcosms and initial samples demonstrated higher number of peaks compared to the exudates amended microcosms (Tukey HSD).

Microcosms treated with algae exudates as well as the control and initial samples demonstrated denitrification rates that were below detection levels. The same was observed for headspace samples collected periodically from the same microcosms.
Fig. 3.26: Bacterial abundance from DAPI counts of anaerobic microcosms amended with algae exudates and leaf leachates. Chlam= *Chlamydomonas*; Chlor= *Chlorella*; Syned= *Synedra*; AM= mixture of algal exudates; RO= red oak; WH= witch hazel; LfM = mixture of leachates; Ctrl= control (no amendment); Ini= initial counts. Values are means and standard errors.
Fig. 3.27: 16S rRNA gene abundance of bacteria incubated in anaerobic microcosms and amended with algae exudates and leaf leachates. Chlam = *Chlamydomonas*; Chlor = *Chlorella*; Syned = *Synedra*; AM = mixture of algal exudates; RO = red oak; WH = witch hazel; LfM = mixture of leachates; Ctrl = control (no amendment); Ini = initial gene abundance. Values are means and standard errors.
Fig. 3.28: RDA axes plots from redundancy analysis of 16S rRNA gene TRFLP peak relative abundance, in anaerobic microcosms amended with A) algae exudates and B) leaf leachates. Chlam= *Chlamydomonas*; Chlor= *Chlorella*; Syned= *Synedra*; mix = mixture of amendments from the same category (exudate or leachate); Control= no amendment; Initial= initial communities.
Fig. 3.29: Number of peaks of 16S rRNA gene TRFLP profiles from anaerobic microcosms amended with algae exudates and leaf leachates. Chlam = *Chlamydomonas*; Chlor = *Chlorella*; Syned = *Synedra*; AM = mixture of algal exudates; RO = red oak; WH = witch hazel; LfM = mixture of leachates; Control = no amendment; Initial = initial peak number. Values are means and standard errors.
nosZ gene abundance, obtained from qPCR, (Fig. 3.30A) of initial samples, control and algae exudate treated microcosms showed significant differences among each other (P< 0.05, F= 4.81, df = 5, one way ANOVA). Control microcosms had significantly lower nosZ gene abundance compared to microcosms treated with the mixture of algal exudates as well as the initial samples. The experimental microcosms, however, did not demonstrate differences in gene abundance among each other. Initial samples were not significantly different from exudate amended microcosms (Tukey HSD).

Relative abundance of denitrifiers, or nosZ gene abundance as a percentage of overall bacterial (16S rRNA gene) abundance (Fig. 3.30B), was significantly different among algae exudates treatments, control microcosms and initial samples (P< 0.05, F= 13.73, df = 5, one way ANOVA). All amendments, except Synedra exudate, demonstrated a decrease in relative abundance compared to initial samples (Tukey HSD). Values obtained from microcosms treated with Synedra exudate were significantly higher than those treated with Chlamydomonas exudate. There was no significant difference in relative gene abundance between single and mixed treatments. Also, relative denitrifier abundance was similar between the exudates amended microcosms and the control.

Redundancy analysis of nosZ gene TRFLP peaks, demonstrated significant differences in denitrifier community structure among treatments (P< 0.05, F= 2.89, df = 5; Fig. 3.31A) and amendment explained 9% of the variation. Although the Synedra, Chlorella and Chlamydomonas exudate amended communities appeared to cluster together on the RDA axes, those treated with the mixture of exudates were spatially
separated from the single amendments. The control and initial communities were also separated from the exudate amended bacteria on the RDA axes.

Differences were also observed in the number of nosZ TRFLP peak numbers (Fig. 3.32), between exudate treatments and control as well initial communities (P< 0.05, F = 23.29, df = 5, one way ANOVA). As observed from community structure when examined with RDA, the control and initial denitrifier communities had significantly higher peak numbers than the algae exudate amended communities (Tukey HSD). The difference observed in distribution of exudate amended communities on the RDA axes was however not reflected in the number of peaks, which were similar among all the treatments.

Nitrate-N concentrations in the water column of anaerobic microcosms (Fig. 3.33) demonstrated a significant difference before and after incubation. In the microcosms amended with algal exudates, not only did the initial and final concentrations differ, but a difference was also observed amongst the microcosms themselves at the end of the experiment (P< 0.05, F = 291.82, df = 9, one way ANOVA). Nitrate-N concentrations were higher before incubation, than at the end, in all cases, including the control (Tukey HSD). After incubation for 10 days, significantly lower concentration of nitrate-N was present in the microcosm treated with Chlorella exudates compared to the other microcosms. Microcosms treated with algae exudates had higher initial concentrations compared to the control since they were obtained from algae grown in Bristol’s medium having a high nitrate-N concentration.
Fig. 3.30: A) *nos Z* gene abundance and B) relative *nosZ* gene abundance expressed as a percentage of 16S rRNA gene, of bacteria incubated in anaerobic microcosms, and amended with algae exudates and leaf leachates. Chlam = *Chlamydomonas*; Chlor = *Chlorella*; Syned = *Synedra*; AM = mixture of algal exudates; RO = red oak; WH = witch hazel; LfM = mixture of leachates; Ctrl = control (no amendment); Ini = initial samples. Values are means and standard errors.
Fig. 3.31: RDA axes plots from redundancy analysis of *nosZ* geneTRFLP peak relative abundance, in anaerobic microcosms amended with A) algae exudates and B) leaf leachates. Chlam= *Chlamydomonas*; Chlor= *Chlorella*; Syned= *Synedra*; AM= mixture of algal exudates; RO= red oak; WH= witch hazel; LM = mixture of leachates; control indicates microcosms with no DOC amendments and initial refers to communities prior to amendments.
Fig. 3.32: Number of peaks from nosZ gene TRFLP profiles of anaerobic microcosms amended with algal exudates and leaf leachates. Chlam= *Chlamydomonas*; Chlor= *Chlorella*; Syned= *Synedra*; AM= mixture of algal exudates; RO= red oak; WH= witch hazel; LfM = mixture of leaf leachates; Ctrl= control (no amendment); Ini= initial samples. Values are means and standard errors.
Fig. 3.33: NO$_3^-$-N concentration of the water column in anaerobic microcosms before (initial) and after (final) amendment and incubation with algal exudates as well as leaf leachates. Chlam = *Chlamydomonas*; Chlor = *Chlorella*; Syned = *Synedra*; AM = mixture of algal exudates; RO = red oak; WH = witch hazel; LfM = mixture of leaf leachates; Control = no DOC amendment. Values are means and standard errors.
DOC concentration of anaerobic microcosms treated with algae exudates (Fig. 3.34) demonstrated significant differences before and after the experiment as well as among treatments at the end of the experiment (P< 0.05, F= 12.66, df = 7, one way ANOVA). All microcosms, except those treated with *Chlamydomonas* exudates, had lower DOC concentrations at the end of the experiment compared to initial concentrations (Tukey HSD). Also, post- incubational DOC concentration of *Chlamydomonas* exudate treated microcosm was significantly higher than that treated with *Synedra* exudate. DOC concentration in the control microcosms was below detection limit.

In microcosms treated with algae exudates, pH was not only different before and after the experiment, but also among microcosms after incubation (P< 0.05, F= 14.92, df = 9, one way ANOVA, Fig. 3.35). For example, pH of microcosms amended with *Chlorella* exudate as well as the mixture of algae exudates was significantly higher at the end of the incubation period compared to the initial pH (Tukey HSD). Furthermore, pH at the end of the experiment was higher in microcosms amended with exudate of *Chlorella* and the mixed treatment compared to those treated with *Chlamydomonas* and *Synedra* exudates.

*Leaf leachate:* DAPI counts from microcosms treated with leaf leachates (Fig. 3.26) demonstrated significant differences from each other as well as with the control and initial counts (P< 0.05, F= 5.86, df = 5, one way ANOVA). Counts from the red oak,
Fig. 3.34: Dissolved organic carbon concentration of the water column in anaerobic microcosms before (initial) and after (final) amendment and incubation with algae exudates and leaf leachates. Chlam= *Chlamydomonas*; Chlor= *Chlorella*; Syned= *Synedra*; AM= mixture of algal exudates; RO= red oak; WH= witch hazel; LfM = mixture of leaf leachates. Values are means and standard errors.
Fig. 3.35: pH of the water column in anaerobic microcosms before (initial) and after (final) amendment and incubation with algae exudates and leaf leachates. Chlam = *Chlamydomonas*; Chlor = *Chlorella*; Syned = *Synedra*; AM = mixture of algal exudates; RO = red oak; WH = witch hazel; LfM = mixture of leaf leachates, Control = no DOC amendments. Values are means and standard errors.
witch hazel and mixed leachate treated microcosms were similar to initial counts and higher than those from corn leachate and control microcosms (Tukey HSD).

16S rRNA gene abundance, obtained from qPCR measurements (Fig. 3.27), was different among treatments, control and initial samples ($P < 0.05$, $F = 24.74$, $df = 5$, one way ANOVA). The gene abundance from witch hazel treatment was higher than those from red oak and mixed leachate treatments, a trend that was different from that observed for DAPI counts. Corn leachate demonstrated counts that were similar to all the other experimental microcosms. Overall, leachate amended microcosms, except that amended with the mixed leachate, showed higher gene abundance compared to both the control microcosm and initial samples. Microcosms treated with the mixture of leaf leachates had gene abundance similar to the control but higher than the initial samples (Tukey HSD).

Leaf leachate amendments led to a significant difference in community structure (Fig. 3.28 B) determined by relative abundance of 16S rRNA gene TRFLP peaks ($P < 0.05$, $F = 2.85$, $df = 5$) and 43% of the variation was explained by treatment. Treatment with leachate from single leaf species did not lead to a difference in community structure. On the other hand, treatment with the mixture of leachates resulted in bacterial community structure to differ from that of the single treatments. The initial communities and ones from the control microcosms were different from the leachate amended communities but were similar to each other.

Number of TRFLP peaks (Fig. 3.29) differed significantly among treatment, control and initial communities ($P < 0.05$, $F = 10.77$, $df = 5$, one way ANOVA). Bacteria
from the control microcosms and initial samples had similar number of peaks that were higher than red oak, witch hazel and corn treatments (Tukey HSD). No difference in peak number was however observed among treatments, as well as among the mixed leachate treated, control and initial bacterial communities.

Denitrification rates, measured from both headspace and artificial substrates, were below detection limits for these microcosms as well as the control and initial samples.

Leaf leachate amendments demonstrated differences in nosZ gene abundance (Fig. 3.30A) among experimental and control microcosms as well as initial samples (P< 0.05, F= 8.16, df = 5, one way ANOVA). Control microcosms demonstrated lower gene abundance compared to all other treatments, except red oak and witch hazel leachate, while the microcosms treated with the leaf leachates did not differ significantly among each other in terms of nosZ gene abundance (Tukey HSD). The only exception to this was the higher peak numbers of denitrifiers from mixed leachate microcosms compared to witch hazel and red oak treatments. Initial abundance was higher than in control microcosms but similar to other amendments.

Microcosms amended with leaf leachates demonstrated significant differences in relative abundance of nosZ genes (percent denitrifiers; Fig. 3.30B) among treatments and initial samples (P< 0.05, F= 13.51, df = 5, one way ANOVA). Higher percent denitrifiers was found in initial samples compared to all microcosms, including the control, but except the one treated with leachate mixture. Treatment with the mixture of leaf leachates
also led to higher nosZ relative abundance compared to witch hazel treatments. The other treatments did not demonstrate a significant difference in relative abundance of the gene. The leachate amended microcosms also did not demonstrate a significant difference with the control (Tukey HSD).

Denitrifier community structure, or relative abundance of nosZ gene TRFLP peaks, differed significantly among microcosms amended with leaf leachates (P< 0.05, F= 2.38, df = 5; Fig. 3.31B). 13% of the variation in community structure was explained by treatment. Denitrifiers treated with witch hazel and corn leachates appeared to cluster on the RDA axis and were separated from those treated with red oak as well as the mixture of leachates. The control and initial communities appeared similar and were different from the denitrifiers from the other microcosms. However, number of nosZ TRFLP peaks (Fig.3.32) were not different among leachate treatments or with the control and initial communities (P= 0.05, F= 4.45, df = 5, one way ANOVA).

Nitrate concentrations (Fig. 3.33) differed significantly before and after incubation for some microcosms amended with leaf leachates (P< 0.05, F= 6.54, df = 9, one way ANOVA). For example, microcosms amended with corn, red oak and witch hazel leachates did not demonstrate such a difference between initial and final concentrations while those amended with the mixture of leachates as well as the control microcosms demonstrated a significant decrease in nitrate concentration over the experiment (Tukey HSD). There was no significant difference among leachate amended microcosms in terms of post-experimental nitrate concentrations.
Differences were observed between initial and post-experimental DOC concentration of water samples (Fig. 3.34) from anaerobic microcosms amended with leaf leachates, and also among the microcosms at the end of the experiment ($P< 0.05$, $F= 21.65$, df $= 7$, one way ANOVA). However, differences between initial and final DOC concentrations were not observed in the case of microcosms treated with corn leachate (Tukey HSD). Also, corn leachate microcosms had higher DOC concentration at the end of the experiment compared to those amended with red oak, witch hazel and mixed leachates. Witch hazel leachate treated microcosms had higher DOC concentration at the end compared to those amended with leachate from red oak. DOC concentration in the control microcosms was below detection limit.

No difference in pH was observed among microcosms amended with leaf leachates before and after the experiment as well as among microcosms at the end of the experiment ($P= 0.06$, $F= 2.32$, df $= 9$, one way ANOVA, Fig. 3.35).

**DISCUSSION**

In this study, bacterial responses to DOC amendments were examined. Amendments were either labile or recalcitrant compounds; and within each category, single compounds differing in molecular structure as well as mixtures of these were used.
Table 3.4: Summary of results obtained from anaerobic microcosms amended with chemically defined and undefined labile and recalcitrant compounds, as individual treatments and their mixtures, and incubated over a period of 10 days. The results are based on statistical analysis of data obtained from the microcosms using one way ANOVA followed by post hoc Tukey except bacterial community structure in which case redundancy analysis of 16S r RNA gene TRFLP peaks was performed. Only significant differences (P< 0.05) among values have been reported. Glu= glucose, Gly= glycine, Ac = Acetate; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; Chlam= Chlamydomonas; Chlor= Chlorella; Syned= Synedra; RO= red oak; WH= witch hazel, S= significant difference, NS= no significant difference.
<table>
<thead>
<tr>
<th>Labile (algal exudates)</th>
<th>DAPI counts</th>
<th>16S rRNA gene</th>
<th>Community structure</th>
<th>16S rRNA TRFLP peak</th>
<th>DOC</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single treatments</td>
<td>NS</td>
<td>NS</td>
<td>Glu, Ac, NS and S from Gly</td>
<td>NS</td>
<td>Final&lt; initial, among treatments NS</td>
<td>Final&gt; Initial, treatments NS</td>
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<tr>
<td>Single vs. Mixed treatments</td>
<td>NS</td>
<td>Mix&gt; Ac</td>
<td>Mix and Gly S</td>
<td>NS</td>
<td>Final&lt; initial, single and mix NS</td>
<td>Final&gt; initial mix, single and mix NS</td>
</tr>
<tr>
<td>Single treatments</td>
<td>SA, VA&gt; CA</td>
<td>NS</td>
<td>VA, CA S from SA</td>
<td>NS</td>
<td>Final&lt; Initial, treatments NS</td>
<td>Final&gt; Initial, treatment-s NS</td>
</tr>
<tr>
<td>Single vs. Mixed treatments</td>
<td>SA, VA &gt; mix</td>
<td>NS</td>
<td>Mix ans SA S</td>
<td>Mix&gt; single</td>
<td>Final&lt; Initial, single and mix NS</td>
<td>Final&gt; initial mix, single and mix NS</td>
</tr>
<tr>
<td>Single treatments</td>
<td>NS</td>
<td>Chlam&gt; Syned</td>
<td>Chlam S from others</td>
<td>NS</td>
<td>Final&lt; initial, except Chlam, latter&gt; Syned</td>
<td>Final&gt; Initial for Chlam</td>
</tr>
<tr>
<td>Single vs. Mixed treatments</td>
<td>NS</td>
<td>NS</td>
<td>Mix and Syned, Chlam S</td>
<td>NS</td>
<td>Mixed final&lt; initial, single vs. mixed NS</td>
<td>Final&gt; initial for mix, Mix&gt; Syned, Chlam</td>
</tr>
<tr>
<td>Single treatments</td>
<td>RO, WH&gt; corn</td>
<td>WH&gt; RO</td>
<td>NS</td>
<td>NS</td>
<td>Final&lt; initial except corn, corn&gt; other, WH&gt; RO</td>
<td>NS</td>
</tr>
<tr>
<td>Single vs. Mixed treatments</td>
<td>Mix&gt; corn</td>
<td>WH&gt; mix</td>
<td>Sl</td>
<td>NS</td>
<td>Final&lt; initial for mix, corn&gt; mix</td>
<td>NS</td>
</tr>
<tr>
<td>Recalcitrant (leaf leachates)</td>
<td>NS</td>
<td>NS</td>
<td>Labile (algal exudates)</td>
<td>Recalcitrant</td>
<td>Labile (algal exudates)</td>
<td>Recalcitrant</td>
</tr>
</tbody>
</table>

**Chemically defined vs. Recalcitrant**

*NS* indicates no significant difference.
Table 3.5: Summary of results obtained for denitrifiers (based on characteristics of *nosZ* gene) from anaerobic microcosms amended with chemically defined and undefined labile and recalcitrant compounds, as individual treatments and their mixtures, and incubated over a period of 10 days. The results are based on statistical analysis of data obtained from the microcosms using one way ANOVA followed by post hoc Tukey except denitrifier community structure in which case redundancy analysis of *nosZ* gene TRFLP peaks was performed. Only significant differences (P< 0.05) among values have been reported. Glu= glucose, Gly= glycine, Ac = Acetate; CA= coumaric acid; SA= syringic acid; VA= vanillic acid; Chlam= *Chlamydomonas*; Chlor= *Chlorella*; Syned= *Synedra*; RO= red oak; WH= witch hazel, S= significant difference, NS= no significant difference.
<table>
<thead>
<tr>
<th>Chemically defined</th>
<th></th>
<th>nos Z gene abundance</th>
<th>Percent denitrifier</th>
<th>Denitrifier community structure</th>
<th>nos Z TRFLP peak number</th>
<th>Denitrification rates</th>
<th>NO₃ - N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labile</td>
<td>Single treatments</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>Ac&lt; glu, gly</td>
<td>NS</td>
<td>Final&lt; Initial, among treatments NS</td>
</tr>
<tr>
<td></td>
<td>Single vs. Mixed treatments</td>
<td>NS</td>
<td>NS</td>
<td>Mix S from gly, ac</td>
<td>Ac&lt; mix</td>
<td>Glu, gly&gt; mix</td>
<td>Final&lt; initial for mix, single and mix NS</td>
</tr>
<tr>
<td></td>
<td>Single treatments</td>
<td>NS</td>
<td>NS</td>
<td>Difference not explained by treatment</td>
<td>NS</td>
<td>NS</td>
<td>Final&lt; Initial, among treatments NS</td>
</tr>
<tr>
<td>Recalcitrant</td>
<td>Single vs. Mixed treatments</td>
<td>NS</td>
<td>NS</td>
<td>Difference not explained by treatment</td>
<td>NS</td>
<td>Single&gt; mix</td>
<td>Final&lt; initial for mix, single and mix NS</td>
</tr>
<tr>
<td>Labile (algal exudates)</td>
<td>Single treatments</td>
<td>NS</td>
<td>Syned&gt; Chlam</td>
<td>NS</td>
<td>NS</td>
<td>Below detection</td>
<td>Final&lt; initial, Chlor &lt; others</td>
</tr>
<tr>
<td></td>
<td>Single vs. Mixed treatments</td>
<td>NS</td>
<td>NS</td>
<td>Mix and single S</td>
<td>NS</td>
<td>Below detection</td>
<td>Final&lt; initial for mix, single and mix NS</td>
</tr>
<tr>
<td>Chemically undefined</td>
<td>Single treatments</td>
<td>NS</td>
<td>NS</td>
<td>WH and corn different from RO</td>
<td>NS</td>
<td>Below detection</td>
<td>NS</td>
</tr>
<tr>
<td>Recalcitrant (leaf leachates)</td>
<td>Single vs. Mixed treatments</td>
<td>Mix&gt; WH, RO</td>
<td>Mix&gt; WH</td>
<td>S</td>
<td>NS</td>
<td>Below detection</td>
<td>Final&lt; initial for mix, single and mix NS</td>
</tr>
</tbody>
</table>
Overall, impacts of amendments varied based on the bacterial response variables measured and on the nature of the DOC treatment. For example, under aerobic conditions, while treatment with labile compounds such as glucose, glycine and acetate, as well as their mixture, led to differences in bacterial community structure, this was not always the case for recalcitrant treatments. On the other hand, treatment with algae exudates and leaf leachates led to differences in bacterial community structure in anaerobic but not in aerobic microcosms. Moreover, while denitrification rates differed between single and mixed treatments for amendments with defined chemistry, the rates remained below detection after treatment with algae exudates and leaf leachates. Most studies compare bacterial response among different categories of DOC, such as labile or low molecular weight versus recalcitrant or high molecular weight compounds, and response within each category are often generalized based on one or two compounds (Covert and Moran, 2001; Young et al., 2004; Romani et al., 2006; Schlief and Mutz, 2007; Khodse and Bhosle, 2011). The current study demonstrated that bacterial response to DOC amendments is more complex, requiring detailed investigation, and cannot be generalized even when the compounds belong to the same general category (such as labile or recalcitrant).

The amendments used in this study were broadly divided into chemically defined and undefined categories. Chemically defined DOC included both labile and recalcitrant compounds. While glucose, glycine and acetate are commonly used as sources of labile carbon (Gruber et al., 2006; Schlief and Mutz, 2007; Thingstad, 2008; Johnson and Tank, 2009a, b), the recalcitrant compounds used in this study, coumaric, syringic and vanillic
acid are less widely used (Alvarez-Rodríguez et al., 2003; Goodman, 2011). These recalcitrant compounds are derivatives of lignin and contain phenolic groups (Stalikas, 2007) which are considered to be recalcitrant to bacteria (Jassey et al., 2011). Chemically undefined amendments were mixtures of compounds from natural sources and these were differentiated between based on functional groupings (Wallace et al., 2008). Analysis of fractions belonging to different functional groupings, separated by ion chromatography, revealed differences between leaf leachates and algae exudates. As expected, leaf leachates had a higher proportion of humic substances compared to algae exudates. Hence, exudates were considered as more labile DOC sources in the current study, compared to leaf leachates.

Bacterial abundance, determined by DAPI counts, differed among amendments with a few exceptions. Abundance was similar among chemically defined labile compounds in anaerobic microcosms and among algae exudates in aerobic microcosms. In the anaerobic microcosms treated with chemically defined labile compounds, all amendments led to a similar increase in bacterial numbers, compared to the initial. While both aerobic and anaerobic microcosms from chemically defined labile treatments demonstrated a decrease in DOC concentration of the water column after the experiment, revealing use of these organic compounds, anaerobic microcosms had lower average final concentrations (4.1 ± 0.82 mgC/L) compared to the aerobic ones (6.12 ± 0.54). Thus for the anaerobic microcosms, DOC concentration may have been a limiting factor overshadowing the effect of DOC quality on bacterial abundance.
In addition, treatment with algae exudates did not stimulate bacterial growth as well based on comparison of bacterial numbers to initial values. A decrease in DOC concentration was also observed in these microcosms at the end of the experiment. Average DOC concentration (apprx. 2.5 mgC/L) was half that of the initial (5 mgC/L) at the end of the experiment and in this case too, it may have been a limiting factor for bacterial growth. In anaerobic microcosms, decreases in DOC concentration occurred to a lesser extent (final DOC concentration was an average of 4 mgC/L). Other studies have also demonstrated DOC concentration can be an important factor determining bacterial abundance (Pomeroy and Wiebe, 2001; Eiler et al., 2003).

In contrast, bacterial abundance determined based on 16S rRNA gene abundance demonstrated trends different from DAPI counts. Abundance was generally similar among treatments within each category with a few exceptions. In cases where differences were observed, they were mostly between single and mixed treatments; for example, acetate amended anaerobic microcosms demonstrated a difference with the labile mixture. Such discrepancies in bacterial abundance between the two methods may arise due to several reasons. Most importantly, the efficiency of detection of the target is different; in other words, the percent recovery of DNA from a given sample may be much lower than the percent recovery and detection of whole cells. Also, DAPI counts are not subject to PCR bias, which is the case for estimation of 16S rRNA gene abundance by qPCR (Acinas et al., 2005). In addition, the reproducibility rates of C, values from quantitative PCR is not as high, compared to the repeatability of fluorescent enumeration techniques (Mutlu and Güven, 2011). Although DAPI counts are subject to manual errors
as well, whole cell counts are free from the above mentioned caveats associated with PCR-based molecular techniques. This is in agreement with a study by Castillo et al. (2006) that showed discrepancies between DAPI counts and 16S rRNA gene abundance, although the authors observed higher abundance from 16S rRNA qPCR compared to DAPI counts, while the opposite trend was observed in this study.

Bacterial community structure was compared among treatments, based on relative abundance of 16S rRNA gene TRFLP peaks, as well peak numbers representing genetic variants. While some treatments resulted in structural differences, others did not. Most of the aerobic microcosms did not demonstrate difference among treatments except for those treated with chemically defined labile compounds. Differences were observed among most single-compound treatments, as well as between single and mixed treatments, in these microcosms. Likewise, anaerobic communities demonstrated difference in structure among single treatments, as well as between single and mixed treatments, for this category. Differences in bacterial community structure with different labile carbon sources possibly arises due to competitive interactions among bacteria for these readily bioavailable compounds (Docherty et al., 2006). The number of genetic variants (peak numbers), however, did not differ much among treatments except in aerobic microcosms where treatment with acetate showed higher peak numbers than the control. Thus, differences in community structure were largely due to relative abundance of taxa differing among treatments and not due to changes in “diversity”.
Recalcitrant treatments led to structural differences among communities in anaerobic but not aerobic microcosms. However, differences in structure observed in anaerobic microcosms were only between syringic acid and all the other amendments. When comparing labile and recalcitrant amendments, the more modest differences among the recalcitrant compound treatments may be a result of lesser competition among bacterial communities owing to the complexity of the recalcitrant molecules. Less competition and lower bioavailability of recalcitrant compounds may then lead to slow growing, stable communities that possess similar capabilities of utilizing these compounds. It is also possible that utilization of these compounds is made feasible by co-occurrence of certain taxa, such that breakdown of these compounds by some taxa possessing the necessary enzymes allows for their uptake by others (Allison, 2005). As a result, recalcitrant treatments of all kinds result in these similar co-occurring communities. This was in agreement with number of peaks as well, which did not differ much among recalcitrant treatments, except in a few cases. For example, coumaric acid treatments in aerobic microcosms and syringic acid treatments in anaerobic ones resulted in higher peak numbers but the other recalcitrant compounds did not result in any such differences.

Lack of difference in bacterial community structure among algal exudates treatment in aerobic microcosms, may have been observed due to DOC concentration becoming a limiting factor. On the other hand, final DOC concentration in the anaerobic microcosms were much higher than aerobic microcosms and differences in community structure among single treatments as well as between single and mixed treatments were
observed in this case. Slower utilization of DOC in the anaerobic microcosms may have prevented concentrations from becoming limiting, leading to differences in bacterial community structure. Number of peaks from 16S rRNA gene analysis, on the other hand, did not demonstrate a difference in either the aerobic or anaerobic microcosms. Hence, relative abundance, rather than the number of operational taxonomic units (OTUs), was mostly responsible for differences in community structure in anaerobic microcosms. Although species specific associations of bacteria with algae have earlier been observed (Sapp et al., 2007), the current study takes a more direct approach at demonstrating the effects of exudates from different algal species on bacterial communities. Additionally, the study suggests that while exudate from different algae can result in differential bacterial response, consideration of DOC concentration may also be important.

For leaf leachates, community structure differed between single and mixed treatments for anaerobic microcosms but no difference among treatments was observed for aerobic microcosms. DOC concentration did not decrease significantly during the experiment for the aerobic microcosms while it did for the anaerobic ones. In this case, bacterial communities may not have been able to uptake DOC from the leachates as efficiently in the aerobic microcosms, as in the anaerobic microcosms. However, even in the anaerobic microcosms, the single leachate treatments resulted in similar communities. As has been observed from DOC fractionation of leaf leachates before, the proportion of humic substances (AHS fraction) was much higher than that of algae exudates and comprised 33% of the total DOC on average. Although the leachates demonstrated differences in the non-humic fractions, the proportion of humic substances was similar
among the different leachate treatments. DOC sources with similar level of complexity may have then led to growth of a certain group of bacteria specialized for the uptake of these compounds. On the other hand, growth of bacteria in a higher degree of chemical complexity, as was the case of the mixture compared to the single leachate treatments, may have required a greater extent of specialization in community structure leading to differences from single treatments. Wu et al. (2009), in a similar study using bacteria from the same stream examined here, demonstrated no significant difference between single and mixed leachate treatments. This may have been because they studied bacterial communities from only aerobic microcosms, while in the present study, differences among leachate types were only observed for anaerobic microcosms.

Differences in denitrification rates were more apparent between single and mixed treatments than among single treatments. More specifically, mixed treatments, both labile and recalcitrant, resulted in lower denitrification rates compared to single treatments. This was not only observed in rates measured from artificial substrates at the end of incubation, but also from periodic headspace samples. Although in the case of recalcitrant treatments, it can be rationalized that a mixture of such compounds with high complexity in chemical structure was not conducive to denitrification, as has been observed in other studies (Dendooven et al., 1996; Dodla et al., 2008), lower rates in microcosm amended with the mixture of labile compounds was contrary to expectations. This is because, in other cases, it has been demonstrated that labile compounds, such as glucose and glycine, enhance bacterial denitrification (Swerts et al., 1996; Dendooven et al., 1996; Gregorich et al., 2006), and hence it is expected that a mixture of such compounds will lead to
higher denitrification rates compared to separate amendments with each by providing resources broadly suitable for denitrifiers. When denitrification rates were followed over time, using glycine and the mixture of labile compounds, the mixture demonstrated low but detectable rates from the fourth day of incubation until the end, while for glycine, the rates were detectable and much higher than the mixture at the end of the 10 day incubation period. This suggests that the molecular heterogeneity of the mixture led to its gradual and steady utilization over time by denitrifiers, lesser heterogeneity of single treatments led to sporadic utilization. Other factors, i.e., final DOC or nitrate concentrations, did not provide an explanation for such an observation since they did not differ among microcosms at the end of the experiment, and were lower than the initial concentrations. For algae exudates and leaf leachate treatments, initial denitrification rates were below detection and the compounds were not successful in enhancing denitrification rates to detection levels. This may have been a result of lower initial DOC concentrations used for these microcosms, compared to the ones treated with chemically defined treatments.

Denitrifier community structure (from analysis of the nosZ gene via TRFLP) was different among single treatments as well as between the single treatments and mixture, with few exceptions, for chemically defined labile treatments. Surprisingly, although treatment with glucose resulted in higher denitrification rates compared to the mixture of labile treatments, bacterial communities from the two treatments clustered together on the RDA axes. Community structure from glycine and acetate treatments however differed from each other as well as the other treatments. The similarities observed in
denitrification rates of acetate and the other treatments, including the mixture, were not reflective of community structure. This indicates that although differences in community structure, like that of glycine with the mixture, may be responsible for differences in denitrification rates, it does not always hold true. Similar communities from the glucose and mixed treatments demonstrated different denitrification rates. This also indicates that the communities that were able to utilize glucose also grew more successfully in the mixture compared to the other communities. In the mixture, competition for the labile compounds from the others may however have resulted in denitrification rates that were lower compared to treatment with only glucose. Also, differences in community structure among the single treatments did not translate to differences in denitrification rates. This suggests that different communities were equally successful in utilizing the labile carbon sources for denitrification, administered separately. While studies by Rich et al. (2003), Kjellin et al. (2007), Phillipot et al. (2009) and Peralata et al. (2010) have demonstrated links between structure and function of denitrifiers, no such relationship was observed in this case, like some others (Rich and Myrold, 2004; Enwall et al., 2005; Dandie et al., 2008; Ma et al., 2008; Attard et al., 2011).

Community structure of denitrifiers was also not related to function in the case of chemically defined recalcitrant treatments. Although differences were observed between the denitrification rates of single and mixed treatments, community structure remained similar among all except the syringic acid treatment. The difference may have been reflective of the number of genetic variants that were higher for the syringic acid treatment than the others. This comparative homogeneity in structure supports the earlier
prediction that recalcitrant compounds led to the growth of relatively stable communities that possess similar capabilities to utilize these complex molecules. The denitrification potential among these communities then differed based on the level of complexity of the source, with the mixture being the most complex and hence demonstrating the least denitrification rates.

Disagreement between structural and functional aspects was also observed when nosZ gene abundance as well as percent denitrifiers were considered, which were similar among all amendments. This was true for both the labile and recalcitrant treatments, even when differences were observed between single and mixed treatments in denitrification rates. While a study by Hallin et al. (2009) demonstrated a strong linkage between denitrifier abundance and denitrification rates, the current study does not reveal this same relationship. The two studies however differ greatly in terms of experimental conditions. The study by Hallin et al. (2009) was carried out in fertilized and unfertilized experimental agroecosystems with and without crops. The study even suggested that application of fertilizers affected denitrifier abundance. In contrast, in the current study, initial NO₃⁻-N and DOC concentrations were similar among all microcosms and the only differences were in the quality of carbon amendment. Also, methodological disadvantages associated with qPCR, as has been previously discussed (Acinas et al., 2005; Castillo et al., 2006; Mutlu and Güven, 2011), may prove to be a stumbling block in arriving at conclusions with regards to relationships between denitrifier abundance and activity.
Although not much can be said about the structure-function relationship of denitrifiers for the algae exudates and leaf leachate treatments, due to undetectable rates of denitrification, differences were observed in community structure. The number of genetic variants remained similar in all cases. The single treatments were mostly similar among each other, with a few exceptions, and were different from the mixed treatments. In spite of differences in some of the DOC fractions between *Synedra* and *Chlorella* exudates, the community structure looked similar. On the other hand, although *Chlorella* and *Chlamydomonas* exudates had similar proportions of the DOC fractions, the community structures were different. It was also observed that final DOC concentration was not significantly different from the initial concentrations in the anaerobic microcosms treated with *Chlamydomonas* exudates and was higher than final concentrations of the other microcosms. This indicates that differences in community structure may arise due to differential uptake and utilization of the DOC. Further, treatment with *Chlamydomonas* exudate resulted in similar community structure as for the mixed exudate treatments. This suggests that bacterial communities growing in the presence of *Chlamydomonas* exudate were able to grow more successfully in mixtures, compared to the ones from the other exudate treatments.

Leaf leachate treatment also led to differences in the denitrifier community. Based on similarities and differences in DOC fractions, treatments with red oak and corn leachates were expected to result in similar communities that differed from that of witch hazel. However, in this case, witch hazel and corn leachates resulted in similar communities that were different from that of red oak. The final DOC concentration of
witch hazel and corn were also higher than that of red oak. Thus, differential uptake of
DOC may have again been responsible for such differences. Community structure of
bacteria treated with the mixture of leachates was different from the single treatments.
This indicates that a different group of bacteria are able to utilize the complex mixture of
leaf leachates compared to the ones utilizing leachates from single leaf species.

Unlike the chemically defined treatments, differences were observed in \textit{nosZ} gene
abundance and percent denitrifiers among treatments with algae exudates and leaf
leachates. Difference in the percent denitrifiers was observed between single treatments
for algae exudates, namely \textit{Synedra} and \textit{Chlamydomonas} exudates. Such differences
coincided with differences in community structure as well and were a result of
differences in 16S rRNA but not the \textit{nosZ} gene abundance. This indicates that treatment
with algae exudates led to differences in the overall abundance of anaerobic bacteria but
did not have any effect on denitrifier abundance. On the other hand, percent denitrifiers
were higher in the mixed leachate treatment compared to witch hazel leachate. This was
reflective of differences in both 16S rRNA and \textit{nosZ} gene abundance. Thus, leaf
leachates, unlike algae exudates, led to differences in both denitrifier and overall
community abundance.

Overall, heterogeneity of DOC led to differences in bacterial community
responses. While treatment with chemically defined labile compounds led to differences
in community structure of both aerobic and anaerobic bacteria, the response to other
treatment types varied. Chemically defined labile treatments also led to differences in
denitrification rates; lower rates in the mixtures, compared to the single treatments, may have resulted from resource use for other similar, processes such as DNRA, for the readily available carbon sources. While the mixture of labile compounds may have adequately supported such alternative processes simultaneously, leading to steady but denitrification rates throughout the incubation period, single compounds may have supported one or the other process at a point of time, due to which they demonstrated sporadic rates of denitrification. Hence at the end, denitrification rates of labile mixtures appeared lower than that of the single labile treatments. Communities able to utilize glucose, unlike other single treatments, were similar to the ones growing in the mix and different from the other single treatments. Denitrification rates were, however, similar among all single treatments. This indicates that different bacteria adapted similarly, with respect to denitrification rates, among single DOC treatments. In relation to single and mixed treatments, communities similar to the ones growing with glucose were better able to utilize the mixture of labile compounds for denitrification, compared to those from the glycine and acetate treatments. Chemically defined recalcitrant treatments did not lead to differences among aerobic communities and for anaerobic microcosms, only a single treatment (coumaric acid) resulted in bacterial communities different from the others. This can possibly be attributed to lower bioavailability of these compounds, compared to labile compounds, leading to lesser competition for carbon source and more stable communities specialized for the uptake of such compounds. Denitrification rates were higher with single compounds than the mixture and were not related to differences, or lack thereof, in the denitrifier communities. nosZ gene abundance as well as percent
denitrifiers were also not related to differences in community structure or denitrification rates. Although algae exudates and leaf leachates did not stimulate denitrification, they led to differences in community structure, nosZ abundance and percent denitrifiers. Thus, differences even within the same category of DOC compounds can lead to differences in bacterial community structure and denitrification, but structure is not necessarily related to function.
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CHAPTER 4

Role of dissolved organic carbon source in determining bacterial community structure and function in a lotic and lentic ecosystem

ABSTRACT

Autochthonous and allochthonous sources of dissolved organic carbon (DOC) influence bacterial community structure and function but not much is known about the influence of differences in proportion of DOC input on bacterial response in ecosystems that are longitudinally connected. In this study, differences in the predominant DOC source and its effect on bacterial communities were examined in a river-reservoir system. Three sites each in the river and reservoir were sampled. Relationships to other environmental variables, such as temperature, dissolved oxygen (DO) and nutrient concentration, were also examined. Temporal variations in environmental factors as well as bacterial responses were studied to determine if differences among and within sites varied over time. Relative dominance of DOC source was determined from the color: chl a
(chlorophyll a) ratio (color indicates absorbance at 440nm), which differed significantly between the river and reservoir sites. Attributes of the bacterial community examined were: abundance (DAPI counts), respiration, productivity, growth efficiency (BGE) and community structure. Bacterial respiration (BR) was determined by changes in oxygen concentrations of water incubated for 24 hours in the laboratory. Bacterial productivity (BP) was measured by uptake of $^3$H-leucine and BGE was calculated as BP/BP+BR.

Bacterial community structure was determined from terminal restriction fragment length polymorphism (TRFLP) analysis. Bacterial productivity was higher in the reservoir than in the river but only on certain dates. Differences in productivity were also observed among reservoir sites, but not among the river sites, on all occasions except one. Unlike productivity, other bacterial variables were not different among sites at any point of time. Relationships with DOC concentration and source were apparent in most cases, except for bacterial respiration which was related only to temperature. While color: chl a was negatively related to bacterial abundance, the relationship between chl a and BGE was positive. BP also demonstrated a positive relationship with chl a. However, such relationships were better able to explain temporal variation in bacterial responses within each ecosystem rather than differences between the river and reservoir ecosystems. For example, while color: chl a was higher in the reservoir than the river sites on 5-October, 2011, no significant difference in BGE was observed between the ecosystems. Instead, within each ecosystem, BGE was higher on 5-October, 2011 compared to most other dates. The only exception to this was the relationship between color and BGE. Color was related to bacterial growth efficiency and both were similar between the river and
reservoir sites. Bacterial community structure was also different between the river and reservoir, but these differences were not related to environmental parameters. Also, differences in community structure among sites did not result in functional differences between ecosystems based on bacterial abundance, respiration and growth efficiency. In addition, differences in bacterial productivity did not follow the same trends as in structural differences. To empirically examine DOC impacts, experiments were performed in laboratory microcosms with river and reservoir water inoculated with bacteria from the same source and amended with algae exudate and leaf leachate. Bacterial community structure (TRFLP) and BP ($^3$H- leucine uptake) were measured. Amendments did not lead to differences in community structure. However, bacterial productivity was higher with leaf leachate in river water microcosms and with algae exudates in reservoir water. Thus, proportional differences between allochthonous and autochthonous inputs did not explain differences in bacterial community structure or function, but based on the relationship between color and BGE, terrestrial inputs may play a role in determining bacterial growth. Results from the laboratory experiment indicated that quality of DOC, rather than proportional differences in input, may play a more important role in determining bacterial responses. Difference in bacterial structure between the river and reservoir ecosystems was not linked to functional differences.
Introduction

Autochthonous and allochthonous dissolved organic carbon (DOC) are important environmental determinants of bacterial community structure and function (Kritzberg et al., 2004, 2006) but bacterial responses vary among ecosystems (Cole et al., 2006; Pohlon et al., 2010; Hanson et al., 2011). For example, while some studies have demonstrated autochthonous carbon to be more important than allochthonous sources in lakes (Kritzberg et al., 2004, 2005; Maki et al., 2010), others have shown that bacteria in lake ecosystems can be dependent on allochthonous DOC as well (Jansson et al., 2007, 2008; Berggren et al., 2010; Maracelli et al., 2011). In lotic ecosystems, on the other hand, even though there is a general consensus that allochthonous DOC from riparian vegetation plays an important role in determining bacterial community structure and function in many streams (Tank et al., 2010; Pollard and Ducklow, 2011), some studies have shown that autochthonous sources can also have an influence on bacterial responses in these ecosystems (Gao et al., 2004; Rier et al., 2007; Pohlon et al., 2010; Risse-Buhl et al., 2011).

Bacterial responses to variation in DOC type differ based on the metabolic capabilities of different taxa (Henssen and Tranvik, 1998; Cottrell and Kirchman, 2000; Pace et al., 2007) and niche partitioning can occur among bacteria in terms of their preference for autochthonous and allochthonous DOC (Jones et al., 2009). In general, while the aromaticity of allochthonous DOC can lead to lower growth efficiency of bacteria (Eiler et al., 2003), autochthonous DOC, which is more labile, can support higher
growth (Biddanda et al., 2001). However, what is labile to one taxon can be highly recalcitrant to another (Amon and Benner, 1996; Jioao and Zheng, 2011; Peter et al. 2011). As a result, both allochthonous and autochthonous DOC can influence bacterial responses in different ecosystems. In spite of general observations that rivers and lakes differ in predominant DOC sources, it is not known whether response of bacterial communities along a riverine -lacustrine gradient is influenced by such differences (Jones, 2010).

The relationship between bacterial community structure and organic carbon utilization is a much debated topic in microbial ecology (Torsvik and Øvreas, 2002; Griffiths et al., 2003; Zak et al., 2003; Philipot and Hallin, 2005; Judd et al., 2006; Fornara and Tilman, 2009; Theuerl and Buscot, 2010). While some studies have shown that bacterial community structure plays a role in determining functional responses to DOC source and quality (Eiler et al., 2003; Carlson et al., 2004; Kirchman et al., 2004; Kritzberg et al., 2006;Judd et al., 2006), others have observed no such relationship (Van Mooy et al., 2004, Lagenheder et al., 2005, 2006 ). Hence when examining the importance of DOC source to bacteria, both bacterial function as well as community structure must be considered.

Bacterial communities are often influenced by variations in environmental parameters occurring over time and responses to DOC source can also change seasonally (Olapade and Leff, 2005; Santmire and Leff, 2006). In temperate ecosystems, temperature as well as DOC and nutrient availability differ with time or among seasons
(Castillo et al., 2004). For systems dominated by autochthonous DOC sources, such temporal variability may translate to changes in DOC quality due to differences in biomass or species of primary producer (Li et al., 2008; Besemer et al., 2005). In systems demonstrating allochthony, DOC concentration and quality are likely to change with time due to changes in the quantity and quality of leaf litter input (Jansson et al., 2000; Lennon and Pffaf, 2005). If DOC source plays an important role in influencing bacterial growth, such temporal changes will result in differential patterns of response over time.

In this study, the differences between a reservoir and river ecosystem were investigated in relation to proportions of autochthonous and allochthonous DOC sources and its influence on bacterial community structure and growth. A river is usually considered to be dominated by allochthonous DOC sources and a lacustrine ecosystem by autochthonous sources. In the current study, a lacustrine system with high primary production fed by a river with lower algal biomass was examined. It was hypothesized that if the proportion of DOC source (allochthonous to autochthonous) is influential in determining bacterial response, then differences in such proportions between the riverine and lacustrine sites will be accompanied by differences in bacterial community structure and function, in spite of their longitudinal connection. It was also predicted that such differences would be observed over time, as long as the dominant DOC source in the two ecosystems differed. The uniqueness of the study lies in comparing bacterial responses of two longitudinally connected ecosystems in relation to predominant DOC source whereas previous studies have compared separate ecosystems (Sandberg et al., 2004; Cole et al., 2006; Kritzberg et al., 2006; Hanson et al., 2011).
In this study, the color: chl $a$ ratio was used as a relative measure of the proportion of allochthonous and autochthonous DOC source in aquatic ecosystems (Pace et al., 2007; Solomon et al., 2008; Weidel et al., 2008). Color is based on absorption of water samples at 440nm which in turn is a measure of chromophoric dissolved organic matter (CDOM) mainly derived from terrestrial sources (Webster et al., 2008; Jones et al., 2009). Chlorophyll $a$, on the other hand, is an approximate estimation of algal biomass which provides an idea about the potential contribution of autochthonous carbon as a DOC source (Jones et al., 2009; Tsai et al., 2008). The ratio is widely used as it is easily measurable and is a good indicator of autochthony versus allochthony in aquatic ecosystems (Pace et al., 2007; Kritzberg et al., 2006; Webster et al., 2008; Jones et al., 2009).

MATERIALS AND METHODS

Study site

The Cuyahoga River originates in Geauga County, north of Akron-Canton plateau, and ends in Lake Erie in Cleveland (Ohio EPA). The river basin is divided into upper, middle and lower parts. The upper part of the river basin starts from the headwaters and extends until Lake Rockwell a reservoir built on the river in Portage County, Kent, OH. Two more reservoirs, the East Branch and La Due, are upstream of Lake Rockwell. The river basin has both urban and agricultural influence, while parts of
it also flows through wooded areas. Upstream of Lake Rockwell, agricultural influence is seen in the upper reaches of the Geauga County, while the other parts also flows through wooded areas before flowing into Rockwell.

Three sites each in Lake Rockwell and the Cuyahoga River, that feeds the reservoir, were chosen for the study. The three river sites (referred to as R1, R2 and R3 in the current study) were approximately 100m apart from each other and had dense riparian cover of trees, including Oak (*Quercus* sp.), Maple (*Acer* sp.), White Pine (*Pinus strobus*) and Cottonwood (*Populus* sp.). Site R3 (41.2137°N and 81.3055°W) was closest to the reservoir while site R1 (41.2107°N and 81.3042°W) was the farthest upstream. Site R2 (41.2132°N and 81.3068°W) was located in between sites R1 and R3. The river channel was approximately 34 meters wide on average in the regions sampled.

Sites in the reservoir were in the lacustrine zone at: 41.1836°N and 81.3261°W (L1), 41.1842°N and 81.3280°W (L2) and finally 41.1836°N and 81.3205°W (L3) and were approximately 400 m apart. L3 was farthest downstream from the river, being closer to the dam area while L1 although still in the lacustrine zone, was closest to the river. Site L2 was located in between L1 and L3. All the sites in the reservoir experienced high algal growth in the summer which is likely to serve as a good source of autochthonous carbon in these sites.
Sample collection and field measurements

Water samples were collected in triplicate from each site on 23-June, 27-July, 25-August and 5-October in 2011, from 0.5m below the surface using Niskin bottles and stored in 10L acid washed and rinsed Nalgene polyethylene containers (Nalgene, Rochester, NY). Environmental parameters, namely, dissolved oxygen, temperature and conductivity were measured using a YSI 85 sonde (YSI Inc., Yellow Springs, Ohio). Containers were brought back to the laboratory within two hours of sample collection for further analysis. Samples were divided into subsamples for the analyses described below.

Sample processing and laboratory measurements

On return to the laboratory, subsamples in triplicates from the respective water samples, were passed through GF/F (Whatman, Maidstone, UK) and subsequently through 0.22 µm filters (Whatman, Maidstone, UK). A portion of the filtrate was frozen for analysis of nitrate and phosphate, while the rest was acidified (using concentrated HCl) and refrigerated for DOC analysis. Later, nutrient analysis was done using a LachAT Quikchem 800 FLA+ system (LachAT Instruments, Hach Company, Loveland, CO) and DOC concentration was determined using a Shimadzu TOC analyzer (Shimadzu Corporation, Columbia, MD).

The GF/F filters from the above filtration were frozen and used later for extraction of chlorophyll a (chl a). Chl a was extracted overnight from macerated GF/F
filters using magnesium-acetone following the standard U.S. EPA protocol. Fluorescence was measured using Turner TD 700 fluorometer (Turner Designs, Sunnyvale, CA) fitted with daylight mercury white lamp with emission (10-051R) and excitation (10-050R) filters, before and after acidification with 0.5N HCl, to correct for phaeophytin. Chl $a$ was calculated using the following formula:

$$\text{Chl } a \ (\mu g/L) = (F_b - F_a) \times Q,$$

where $F_b =$ fluorescence before acidification, $F_a =$ fluorescence after acidification, $Q = m \times R/ (R-1) \times \text{extraction volume/ filter volume}$

where $m =$ scale factor and $R =$ acid ratio.

Color or absorbance at 440nm is a measure of chromophoric dissolved organic matter (CDOM), an indicator of allochthony (Webster et al., 2008; Jones et al., 2009). Absorbance of water samples at 440nm was measured using a UV/vis spectrophotometer (Beckman Coulter, Inc., Brea, CA) after filtration of subsamples from the respective water samples through 0.45µm filters (Millipore, Billerica, MA) and is hereby referred to as color. All measurements were made in 10cm quartz cells and double distilled deionized water was used as blank. Absorbance values were converted to absorption coefficients as per Cuthbert and del Giorgio (1992).

**Bacterial measurements**

A subsample from each replicate sample was preserved with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO) such that there were triplicates for
each site and thereafter bacteria were enumerated by fluorescent staining with 4’,6-
diamidino-2-phenylindole (DAPI) for 3 minutes. Bacteria in 10 fields per slide were
enumerated via epifluorescence microscopy.

Bacterial respiration was measured as a change in dissolved oxygen (DO)
concentration over 24 hours. Water from each location and date (subsamples from water
collected as described above) were passed through 1.0µm filters (Whatman, Maidstone,
UK) to remove larger organisms. 300ml Wheaton bottles (Wheaton, Millville, NJ) were
filled to the brim with the filtered samples and capped. The sides of the bottle were
tapped to get rid of any air bubbles. Two sets of bottles were prepared, one for
measurement of initial DO concentration and the other for measuring DO after being
stored in dark for 24 hours. DO concentrations were measured by the standard Winkler’s
titration procedure, at 0 and 24 hours, and bacterial respiration rate at each time point was
calculated as:

\[ \text{mg O}_2/\text{L} = (\text{ml titrant}) \times (\text{molarity of thiosulfate}) \times (8000)/ (\text{ml sample water

titrated}) \]

The difference in DO concentrations of the two time points (0 and 24 hours) was used as
a measure of bacterial respiration.

Bacterial heterotrophic productivity was determined from subsamples from each
sample by measuring \(^3\text{H}\)-leucine incorporation (Jorgensen 1992). Formalin fixed controls
were used to correct for non-specific uptake. Each sample was amended with \(^3\text{H}\)-leucine
(New England Nuclear Corporation – DuPont, 50 Ci/mmol specific activity) and
incubated for 1 hour. The incubated samples were then centrifuged for 10 minutes after addition of 50% trichloroacetic acid (TCA, MP Biomedicals, Solon, OH). Next, 5% TCA was added to the pellet and centrifuged for another 10 minutes. $^3$H dpm of each filter was determined by liquid scintillation in a Beckman LS6800 liquid scintillation counter (Beckman Inc., Brea, CA) after adding scintillation fluid to the pellet.

Bacterial growth efficiency (BGE) is an estimate of the amount of new biomass produced by bacteria per unit of carbon assimilated (del Giorgio and Cole, 1998). High values of BGE indicate lower energy allocations to maintenance and carbon utilization and vice versa. BGE was calculated from bacterial respiration (BR) and heterotrophic productivity (BP) as follows:

$$\text{BGE} = \frac{\text{BP}}{\text{BR} + \text{BP}}$$

Bacterial community structure was assessed by terminal restriction fragment length polymorphism (TRFLP) of 16S rRNA gene polymerase chain reaction (PCR) products. DNA was extracted for PCR using the MoBio Power Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA) following the manufacturer’s protocol. PCR was performed on the 16S rRNA gene by using an equimolar mixture of 5'-ACTCCTACGGGAGGCWGC-3' (Eub338F-0-III) and 5'-ACACCTACGGGTGCGWGC-3' (Eub338F-I-II) for the forward primer and 5’-ACGGGCGGTGTACAC-3’ (1392R) for the reverse primer ($W= A$ or $T$; Blackwood et al., 2005). The forward primers were fluorescently labeled with 6-carboxyfluorescein (6-FAM). A 25µl reaction mixture contained GoTaq Flexi DNA polymerase (2.5 U), buffer (1X), MgCl$_2$ (0.5 mM), bovine serum albumin (0.64 mg ml$^{-1}$), deoxynucleoside
triphosphates (0.2 mM each), forward and reverse primers (0.2 µM each) along with template DNA. 30-35 cycles of PCR reaction was carried out in a PTC 200 DNA Engine Cycler (Biorad, Hercules, CA) with the thermal profile being 94°C for 3 min and 30 cycles of 94°C (30 s), 57°C (30 s), and 72°C (1 min 30 s) followed by a final extension of 72°C for 7 min. Five reactions were carried out for each sample and products were pooled before performing gel electrophoresis on 1% agarose gels stained with ethidium bromide to check for amplification and sizes of products. Products were purified using a Qiaquick PCR purification kit (Qiagen, Valencia, CA) and digested with endonuclease HaeIII (2U, New England Biolabs, Ipswich, MA) at 37°C for 18 to 24 hours. T-RFLP analysis was performed at The Ohio State Plant Microbe Genomics Facility using a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) and the results analyzed via GeneMapper 4.0 (Applied Biosystems, Foster City, CA).

**Experiment**

Experimental microcosms were set up using samples collected on 25-August, 2011 to determine the effects of autochthonous (algae exudates) and allochthonous (leaf leachates) sources on bacterial communities under laboratory conditions. Algal exudates were prepared by growing *Chlamydomonas, Chlorella and Synedra* cultures (Carolina Biological Supplies, Burlington, NC) in Bristol’s medium (10 ml NaNO₃, 10 ml CaCl₂, 2H₂O, 10 ml K₂HPO₄, 10 ml KH₂PO₄, 10 ml MgSO₄, 7H₂O, 10 ml NaCl in 940 ml dH₂O) for two weeks prior to setting up the experiment under constant light. Sodium
metasilicate (Na$_2$SiO$_3$) was added to facilitate growth of the diatom *Synedra*. The cultures were then filter sterilized by passage through GF/F filters (Whatman, Maidstone, UK) followed by 0.22µm filters (Whatman, Maidstone, UK). The exudates were mixed together in equal proportions and DOC measured from a subsample using the Shimadzu TOC analyzer (Shimadzu Corporation, Columbia, MD). Leaf leachate was prepared from a mixture of red oak (*Quercus rubra*), witch hazel (*Hamamelis virginiana*), and corn (*Zea mays*) by stirring in 0.027% NaCl overnight in the dark. The leachate obtained was filtered similarly as the algae exudates and DOC was measured from subsamples in a similar manner.

Samples collected from the three river sites were pooled together, filtered through pre-combusted Gelman AE filters (Fisher Scientific, Pittsburgh, PA) and subsequently through 0.22µm polycarbonate filters (Whatman, Maidstone, UK) as per Kritzberg et al. (2006). The same was done with samples collected from the three reservoir sites. 500ml of filtered water was dispensed in 2L beakers in triplicates and incubated overnight in dark with 100ml of GF/D (Whatman Maidstone, UK, nominal pore size 2.7µm) filtered bacterial inoculums obtained from the same source (river or reservoir). The aim of filtration through GF/D filters was to remove bigger particles, like bacterial predators, from the inoculum. Algae exudates and leaf leachate were added to the microcosms to a final concentration of 5 mg/L which was similar to the average DOC concentration of water samples from the study site. The control was also set up in triplicates and consisted of filtered reservoir or river water, as above, to which no DOC was added. The beakers were then incubated in room temperature in the dark for 5 days and river samples were
stirred constantly with Phipps and Bird paddle stirrers (Phipps and Bird, Richmond, VA). At the end of the incubation period, subsamples were collected and preserved for DOC and 16S rRNA gene TRFLP analysis. Subsamples were also used immediately for measurement of bacterial productivity using the same procedure as described before.

**Statistical analysis**

Two way ANOVA followed by post hoc Tukey HSD using JMP statistical software (version 8) was used to determine differences in physicochemical and biological variables among sites, dates and the site by date interaction. The physicochemical variables include temperature, dissolved oxygen, DOC, soluble reactive phosphorus (SRP) and nitrate concentrations as well as color (absorbance at 440nm). Besides chl \(a\) concentrations, biological variables include bacterial abundance, respiration, heterotrophic productivity and growth efficiency.

Multiple linear regression, with stepwise forward and backward selection, was used to determine the relationship between bacterial variables (bacterial abundance, respiration, heterotrophic productivity and growth efficiency) and physicochemical factors color: chl \(a\) ratio, absorbance at 440nm, chl \(a\), SRP, nitrate, DOC and DO concentrations. The values to enter and leave the analyses were 0.100 and 0.05, respectively.

Redundancy analysis was performed, on the relative abundance of 16S rRNA gene TRFLP peaks, to determine the effect of differences in site and associated
physiochemical as well as biological (chl a) parameters on bacterial community structure. Differences in relative peak heights (Hellinger distance) as well as peak presence or absence (Jaccard distance) were considered to determine differences between profiles and analyses were performed using R statistical software (ver. 2.13.2 for Windows). TRFLP relative peak heights were square root transformed prior to analysis.

For the experimental microcosms, differences among amendments in terms of bacterial heterotrophic productivity and DOC concentrations were determined by one way ANOVA followed by post hoc Tukey HSD using the JMP statistical software (version 8). Redundancy analysis, as before, was performed to determine the effect of amendments on variation among TRFLP profiles of the 16S rRNA gene using R statistical software (ver. 2.13.2 for Windows).

RESULTS

Physicochemical Responses

Water temperature (Table 4.1) was significantly higher in the reservoir and than in the river (P<0.05, F= 377.15, df = 15; Tukey HSD). Temperature differed significantly among sampling dates as well (P<0.05, F= 9420.58, df= 5); highest temperatures were on 27-July and lowest on 5-October (Tukey HSD). Water temperature on 23-June and 25-August, 2011 were similar to each other and was in between that of 27-July and 5-
October, 2011. The interaction of site and date was also significant (P< 0.05, F= 24.52, df= 15).

Dissolved oxygen (DO) concentration was significantly different among dates (P<0.05, F= 10.20, df = 3), sites (P<0.05, F= 15.29, df= 5) and the interaction between date and site was also significant (P< 0.05, F= 12.49, df= 15, Table 4.1). DO concentrations on 23-June and 27-July were significantly higher than on 25-August and 5-October, 2011 (Tukey HSD). The reservoir sites had higher DO concentrations, averaging 8.07± 0.15 mgO$_2$/L, compared to the river sites averaging 7.04± 0.07 mgO$_2$/L, at all times other than 5-October, 2011. Only on 5-October, 2011, lower DO concentration was observed in the reservoir sites compared to the river sites. While the river and reservoir sites generally had different DO concentrations, differences among the reservoir sites was evident on 23-June and 5-October, 2011 when two of the reservoir sites (L1 and L2) demonstrated DO concentrations that were significantly higher than the other reservoir site (L3). DO concentration among the river sites was similar throughout.

Dissolved organic carbon (DOC) concentration was significantly different among dates (P< 0.05, F= 53.53, df = 3) and sites (P<0.05, F= 4.79, df= 5) while the site and date interaction was not significant (P= 0.07, F=1.73, df= 15, Table 4.1). DOC concentrations on 23-June and 5-October were similar and significantly lower than that on 27-July and 25- August, 2011. Sites L1 and L2 had similar DOC concentrations to the river sites, while L3, the site farthest away from the river, had higher DOC concentration compared to the river sites. An exception to this was observed on 23-June and 5-
October, 2011 when DOC concentration at L3 was lower than that of the river sites. No difference in DOC concentration was observed within the reservoir or river sites (Tukey HSD).

Soluble reactive phosphorus (SRP) differed significantly among sites (P<0.05, F=72.53, df=5) and dates (P<0.05, F= 16014.87, df=3) with the interaction of site and date being significant (P<0.05, F= 86.35, df=15) as well (Table 4.1). River sites had twice the SRP concentration compared to reservoir sites on 5-October, 2011. At all other times, the concentrations were similar. With respect to dates, while SRP concentrations were similar between 23-June and 27-July, they were higher than that on 25-August which in turn was higher than 5-October, 2011 (TUKEY HSD).

Nitrate concentrations differed significantly with site (P<0.05, F= 9.24, df = 23), date (P<0.05, F= 2510.04, df = 3) as well the site by date interaction (P<0.05, F= 4.9, df = 15, Table 4.1). A similar pattern of change over time, as in SRP concentration, was observed. Nitrate concentration decreased significantly over the sampling period but 23-June concentration was similar to that of 27-July for all sites, thereby decreasing on 25-August and then again on 5-October, 2011. Site R3 demonstrated significantly higher nitrate concentrations compared to all other sites only on 25-August, 2011. On the other sampling dates, nitrate concentration was similar among all sites.
Table 4.1: Mean values (± standard errors) of environmental variables at Lake Rockwell and Cuyahoga River in 2011 on the respective dates Sites L1, L2 and L3 are three different sites in the reservoir while R1, R2 and R3 are three sites in the river flowing into the reservoir. *Temperature, □Dissolved Oxygen, **Dissolved Organic Carbon, †Soluble Reactive Phosphorus.
<table>
<thead>
<tr>
<th>Date</th>
<th>Site</th>
<th>Temp°C</th>
<th>DO mg/L</th>
<th>DOC mgC/L</th>
<th>SRP µgP/L</th>
<th>Nitrate mgN/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-June</td>
<td>R1</td>
<td>22.20±0.00</td>
<td>5.89±0.46</td>
<td>7.12±0.21</td>
<td>71.93±0.03</td>
<td>2.38±0.02</td>
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<tr>
<td></td>
<td>R2</td>
<td>22.30±0.00</td>
<td>7.35±0.03</td>
<td>7.32±0.55</td>
<td>72.07±0.13</td>
<td>2.36±0.02</td>
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<tr>
<td></td>
<td>R3</td>
<td>22.40±0.20</td>
<td>6.28±0.17</td>
<td>6.44±0.13</td>
<td>72.57±0.22</td>
<td>2.32±0.02</td>
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<tr>
<td></td>
<td>L1</td>
<td>23.93±0.07</td>
<td>10.89±0.34</td>
<td>5.63±0.02</td>
<td>71.77±0.57</td>
<td>2.34±0.01</td>
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<tr>
<td></td>
<td>L2</td>
<td>23.97±0.09</td>
<td>10.86±0.53</td>
<td>5.67±0.00</td>
<td>72.03±0.13</td>
<td>2.37±0.01</td>
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<td></td>
<td>L3</td>
<td>23.87±0.17</td>
<td>8.20±0.58</td>
<td>5.35±0.06</td>
<td>71.73±0.07</td>
<td>2.18±0.04</td>
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<tr>
<td>27-July</td>
<td>R1</td>
<td>23.60±0.00</td>
<td>7.03±0.00</td>
<td>10.66±0.87</td>
<td>71.7±0.06</td>
<td>2.42±0.04</td>
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<td>R2</td>
<td>23.37±0.17</td>
<td>6.58±0.01</td>
<td>10.41±0.78</td>
<td>71.83±0.03</td>
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<td>R3</td>
<td>23.90±0.00</td>
<td>6.1±1.49</td>
<td>9.23±0.34</td>
<td>71.87±0.03</td>
<td>2.48±0.24</td>
</tr>
<tr>
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<td>L1</td>
<td>27.87±0.03</td>
<td>9.43±0.24</td>
<td>10.83±1.43</td>
<td>72.2±0.26</td>
<td>2.37±0.00</td>
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<td>L2</td>
<td>27.83±0.03</td>
<td>8.51±0.58</td>
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<td>21.9±0.06</td>
<td>7.71±0.09</td>
<td>7.39±0.19</td>
<td>51.83±0.12</td>
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<td>7.55±0.21</td>
<td>51.87±0.07</td>
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<tr>
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<td>8.97±0.91</td>
<td>51.93±0.03</td>
<td>1.93±0.03</td>
</tr>
<tr>
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<td>24.13±0.03</td>
<td>7.80±0.36</td>
<td>8.36±0.03</td>
<td>52.2±0.32</td>
<td>1.36±0.01</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>24.13±0.03</td>
<td>7.68±0.22</td>
<td>8.88±0.33</td>
<td>52.57±0.19</td>
<td>1.54±0.01</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>24.33±0.12</td>
<td>7.79±0.32</td>
<td>9.78±0.56</td>
<td>51.87±0.07</td>
<td>1.37±0.00</td>
</tr>
<tr>
<td>5-October</td>
<td>R1</td>
<td>12.53±0.09</td>
<td>8.13±0.06</td>
<td>7.43±0.23</td>
<td>28.87±0.88</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>12.43±0.07</td>
<td>8.25±0.04</td>
<td>7.06±0.06</td>
<td>27.7±1.15</td>
<td>0.20±0.00</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>12.6±0.05</td>
<td>7.80±0.09</td>
<td>7.03±0.14</td>
<td>27.33±1.01</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>14.27±0.38</td>
<td>6.80±0.29</td>
<td>7.56±0.24</td>
<td>10.72±0.75</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>14.3±0.25</td>
<td>6.45±0.08</td>
<td>7.35±0.23</td>
<td>11.53±0.20</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>14.2±0.30</td>
<td>4.31±0.16</td>
<td>6.90±0.21</td>
<td>15.57±0.95</td>
<td>0.02±0.00</td>
</tr>
</tbody>
</table>
Color or absorbance of water samples at 440nm (Fig. 4.1) is an approximate indication of allochthony and was significantly different among dates ($P < 0.05, F = 108.9, \text{df} = 3$) but not among sites ($P = 0.1, F = 1.95, \text{df} = 5$). The date by site interaction was, however, significant ($P < 0.05, F = 2.23, \text{df} = 15$). On a particular date, the river and reservoir sites had similar absorbance when compared to each other. In the reservoir, absorbance was higher on 5-October, 2011 compared to 23-June, which in turn was higher than 27-July, and 25-August, 2011, the absorbance being similar on the latter two dates. On the other hand, in the river sites, while 25-August, 2011 demonstrated lower absorbance compared to the other dates, 5-October, 2011 demonstrated the highest absorbance (Tukey HSD).

Chlorophyll a

Chl $a$ (Fig. 4.2) was significantly different among not only sites ($P < 0.05, F = 31.35, \text{df} = 5$) but also dates ($P < 0.05, F = 289.37, \text{df} = 3$), the date by site interaction being significant as well ($P < 0.05, F = 7.60, \text{df} = 15$). In general, chl $a$ concentration was higher in the reservoir than the river, except on 25-August, 2011 when they were similar (Tukey HSD). 27-July, 2011 demonstrated highest concentrations, which then decreased in the order of 23-June, 2011, 25-August and 5-October, 2011.
Fig. 4.1: Absorbance at 440nm, determined by spectrophotometric analysis, in three different sites each on Lake Rockwell (L1, L2 and L3) and the Cuyahoga River (R1, R2 and R3) draining into the reservoir on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. The solid black bars represent river sites while the white bars with black border represent reservoir sites. All values are means and standard errors.
Absorbance at 440 nm (m$^{-1}$)

A

B

C

D
Fig. 4.2: Chl $a$ concentrations, determined by fluorometric analysis, in three different sites each on Lake Rockwell (L1, L2 and L3) and the Cuyahoga River (R1, R2 and R3) draining into the reservoir on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. The solid black bars represent river sites while the white bars with black border represent reservoir sites. All values are means and standard errors.
Color: chl a ratio

Color: chl a ratio (Fig.4.3) was significantly different among dates (P<0.05, F=61.43, df = 3), sites (P< 0.05, F= 12.72, df = 5) and the site by date interaction (P<0.05, F= 14.64, df = 15) was also significant. The ratio was higher on 5-October, 2011 compared to the other dates mainly due to low values of chl a on that date. This was true both for the reservoir and the river sites. The reservoir sites generally demonstrated lower color: chl a ratio compared to river sites, except for similar values on 25-August, 2011, due to higher chl a concentration in the reservoir compared to the river sites.

Microbial responses

Bacterial abundance (Fig. 4.4), based on DAPI counts, differed among sampling dates (P<0.05, F= 29.54, df = 3) but the other effects (sites, site and date interaction) were not significant (Site: P= 0.16, F=8.04, df = 5; Site by date interaction: P= 0.23, F= 2.34, df= 15). Bacterial counts were lowest on 5-October, 2011 (average 2.24± 0.49 X 10^6 cells/ml) and highest on 27-July, 2011 (average 7.87± 0.76 X 10^6 cells/ml). Abundance on 23-June, 2011 was similar to that of 25-August, 2011 and ranged in between. Bacterial abundance was correlated with the color: chl a ratio, temperature, DOC, SRP and nitrate concentrations as determined by multiple regression analysis (P< 0.05, R^2= 0.47, R^2 adj= 0.43). While abundance showed a positive relation with temperature, DOC, SRP and nitrate concentrations, the relationship with color: chl a ratio was negative.
Bacterial respiration (Fig. 4.5) differed significantly among dates (P<0.05, F= 8, df= 3) but not among sites (P= 0.13, F= 0.64, df = 5) and the interaction was not significant (P= 1.34, F= 1.34, df= 15). Bacterial respiration on 5-October, 2011 was an order of magnitude lower than all the other dates (Tukey HSD). Respiration showed a positive relationship with temperature (P< 0.05, R² = 0.10, R² adj= 0.08) but no significant relationship was observed with the other variables (P> 0.05).

Bacterial productivity (Fig. 4.6) differed among dates (P< 0.05, F= 31.54, df = 3), sites (P< 0.05, F= 8.04, df= 5) and the interaction effect was also significant (P<0.05, F= 2.34, df= 15). Productivity on 25-August, 2011 was significantly higher than that on other dates (P< 0.05). Sites L1 and L3 demonstrated higher productivity compared to L2, except on 23-June, 2011, when all the three reservoir sites had similar productivity rates (Tukey HSD). The reservoir sites, except L2, showed higher productivity than the river sites only on 27-July and 25-August, 2011. Multiple regression analysis demonstrated a positive relationship of bacterial productivity to temperature, nitrate and chl a concentrations (P< 0.05, R² = 0.49, R² adj= 0.45).

Bacterial growth efficiency (BGE, Fig. 4.7) was significantly different among dates (P< 0.05, F= 18.04, df = 3) but not among sites (P= 0.51, F= 0.88, df= 5); the site and date interaction also not being significant (P= 0.22, F= 1.33, df = 15). BGE on 25-August and 5-October, 2011 was significantly higher than that on 23-June and 27-July, 2011 (Tukey HSD). Multiple regression results revealed a significant negative
relationship of BGE with SRP concentration, and positive relationship with color and color: chl $a$ ratio ($P<0.05$, $R^2 = 0.46$, $R^2$ adj= 0.42).

Bacterial community structure was significantly different among sites ($P<0.05$; Fig. 4.8) on each date. Community structure in the reservoir sites clustered away from the river sites on the RDA axes. Sites within the river or reservoir also demonstrated differences on each date. On 23-June, 2011, with respect to the RDA axes, sites L2 and L3 were positioned separately from L1, the site closest to the river. In the river, site R2 demonstrated community structure that was different from R1 and R3. On 27-July, 2011 community structure in L3 was different from L1 and L2 and that of R3 was different from R1 and R2. On 25-August, 2011, two of the reservoir sites, L1 and L3 were spatially separated from L2, on the RDA axes. However, no such separation was observed in bacterial communities from the river sites, all of which grouped in the same quadrant of the axes. The same pattern seen on 25-August was repeated for the reservoir sites on 5-October, 2011, except in this case bacterial community structure in R3 was different from R1 and R2.

Temporal differences in community structure were also observed for each site ($P<0.05$, $F= 2.31$, df =23), with some exceptions. At site L1, while bacterial community structure was similar between 25-August and 5-October, 2011, differences were observed among all other dates. At site L2, communities on 23-June and 5-October were similar and different from 27-July and 25-August, 2011. Bacterial communities in site L3 were only similar between 27-June and 25-August, 2011 but differed on all other dates. River
sites also demonstrated such trends, when communities were different among certain
dates while not so among others. In site R1, bacterial community only on 25-August,
2011 was different from the other dates, and no difference was observed among other
dates. At site R2, community structure changed from 23-June to 27-July, remained the
same between 25-July and 27-August, and changed again on 5-October, 2011 such that
these communities were similar to the ones from 23-June. In site R3, community
structures demonstrated similarities only between 23-June and 25-August but were
different among all other dates.

The difference in bacterial communities among sites was not, however, explained
significantly by the physicochemical or biological variables on any of the dates (P>0.05),
as suggested by redundancy analysis. Physicochemical variables considered for the
analysis were temperature, color, color: chl a ratio, DOC, SRP, nitrate and DO
concentrations. Biological variables considered were chl a concentration, bacterial
numbers (determined by DAPI counts), heterotrophic productivity, respiration and
growth efficiency. Redundancy analysis was then performed using relative abundance of
16S rRNA gene TRFLP peaks and the physicochemical, as well as, biological variables.
Results from the analysis suggested that there was no significant relation between
bacterial community structure and physicochemical variables as well as between structure
and biological variables.
Fig.4.3: Ratio of color and chlorophyll $a$ in three different sites each on Lake Rockwell (L1, L2 and L3) and the Cuyahoga River (R1, R2 and R3) draining into the reservoir on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. The solid black bars represent river sites while the white bars with black border represent reservoir sites. All values are means and standard errors.
Fig. 4.4: Bacterial abundance, determined by DAPI counts, in three different sites each on Lake Rockwell (L1, L2 and L3) and the Cuyahoga River (R1, R2 and R3) draining into the reservoir on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. The solid black bars represent river sites while the white bars with black border represent reservoir sites. All values are means and standard errors.
Fig. 4.5: Bacterial respiration, determined by change in dissolved oxygen concentrations over 24 hours, after filtration of the water samples through a 1µm filter. Samples were collected from three different sites each on Lake Rockwell (L1, L2 and L3) and the Cuyahoga River (R1, R2 and R3) draining into the reservoir on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. The solid black bars represent river sites while the white bars with black border represent reservoir sites. All values are means and standard errors.
Fig. 4.6: Bacterial heterotrophic productivity, measured by uptake of $^3$H-leucine, in three different sites each on Lake Rockwell (L1, L2 and L3) and the Cuyahoga River (R1, R2 and R3) draining into the reservoir on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. The solid black bars represent river sites while the white bars with black border represent reservoir sites. All values are means and standard errors.
Fig. 4.7: Bacterial growth efficiency, measured as a ratio of bacterial heterotrophic productivity with the sum of bacterial heterotrophic productivity and bacterial respiration, in three different sites each on Lake Rockwell (L1, L2 and L3) and the Cuyahoga River (R1, R2 and R3) draining into the reservoir on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. The solid black bars represent river sites while the white bars with black border represent reservoir sites. All values are means and standard errors.
Fig. 4.8: RDA plots of relative abundance of 16S rRNA geneTRFLP peaks related to bacterial communities from Cuyahoga River (R1, R2 and R3) and Lake Rockwell (L1, L2 and L3) on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. L1, L2 and L3 are reservoir sites while R1, R2 and R3 are sites in the river.
Numbers of TRFLP peaks are a proximal measure of the number of bacterial genetic variants in a sample. In this study, while the number of TRFLP peaks (Fig. 4.9) was not significantly different among sites (P= 0.05, F= 2.4, df = 5) or dates (P= 0.19, F= 1.63, df= 3) the site by date interaction was significant (P< 0.05, F= 2.65, df= 15). On 27-July, 2011, the number of peaks or genetic variants from sites L2 and L3 in the reservoir were significantly higher than river sites (Tukey HSD).

**Experimental Results**

Experimental microcosms were set up with reservoir and river water amended with leaf leachates and algae exudates and incubated with bacteria from the same source, and no amendment serving as the control. Over the course of the experiment, DOC in microcosms comprising of river and reservoir water (Fig. 4.10A), were significantly lower at the end of the experiment and also differed among microcosms (P< 0.05, F= 324.53, df= 3). For river water microcosms, DOC concentration with algae exudates treatment was significantly higher than the control and leachate amended microcosms at the end of the experiment. In case of the microcosms containing reservoir water and bacteria (Fig. 4.10B), post incubation, those amended with algae exudate demonstrated lower DOC concentration compared to the other microcosms, including the control.

Bacterial heterotrophic productivity differed among microcosms containing river (P< 0.05, F= 232.8, df = 2; Fig. 4.11A) as well as reservoir (P< 0.05, F= 72.54, df = 2; Fig. 4.11B) water at the end of the experiment. For the river water, bacterial productivity
was lower than in the leaf leachate amended microcosms (Tukey HSD). Bacterial productivity was higher in both amendments compared to the control. Microcosms containing reservoir water demonstrated highest bacterial productivity with algae exudate amendments, while leaf leachate treatment resulted in values lower than even the control.

In spite of differences in productivity, treatment did not significantly contribute to differences in bacterial community structure for both the river (P= 0.66, F= 0.92, df= 2; Fig 4.12A) and reservoir water (P= 0.88, F= 0.75, df= 2, Fig. 4.12B) containing microcosms. A similar lack of difference was observed in TRFLP peak numbers, or number of genetic variants, between the two treatments (algae exudate and leaf leachate) for microcosms containing river (P= 0.13, F= 4.5, df = 2; Fig. 4.13A) as well as reservoir water (P= 0.27, F= 2.1, df = 2; Fig. 4.13B).

**DISCUSSION**

Bacterioplankton community structure and growth responses were compared between a riverine and lacustrine ecosystem differing in the dominant source of DOC. This difference in dominant DOC source was deduced from differences in the color: chl \( a \) ratio between the two ecosystems. The ratio was higher in the river, compared to the reservoir, in the summer months (23-June; 27-July and 25-August, 2011) while it was similar in fall (5-October, 2011). This temporal difference between summer and fall was mainly reflective of higher chl \( a \) concentration in the reservoir in summer compared to fall. Such a trend was expected because in summer months higher algal growth
Fig. 4.9: Number of peaks from 16S rRNA gene TRFLP profiles of bacterial communities from Cuyahoga River (R1, R2 and R3) and Lake Rockwell (L1, L2 and L3) on A) 23- June, B) 27- July, C) 25- August and D) 5- October in the year 2011. The solid black bars represent river sites while the white bars with black border represent reservoir sites. All values are means and standard errors.
Fig. 4.10: DOC concentration of the water column before and after the experiment performed using microcosms (in triplicates) containing A) river and B) reservoir water, along with bacteria from the respective sources, amended with algae exudates and leaf leachates (mixture of red oak, witch hazel and corn). (mixture of exudates from *Chlamydomonas*, *Chlorella* and *Synedra*). Black bars represent DOC concentrations before the experiment while white bars are post- experimental concentrations. Values are means and standard errors.
Fig. 4.11: Bacterial heterotrophic productivity, measured by uptake of $^3$H- leucine, measured from the experimental microcosms set up in triplicates with A) river and B) reservoir water and amended with leaf leachates (mixture of red oak, witch hazel and corn) and algae exudates (mixture of exudates from *Chlamydomonas, Chlorella* and *Synedra*). Values are means and standard errors.
Fig 4.12: RDA plots of relative abundance of 16S rRNA gene TRFLP peaks related to bacterial communities treated with algae exudates (mixture of exudates from *Chlamydomonas, Chlorella* and *Synedra*) and leaf leachates (mixture of red oak, witch hazel and corn) in experimental microcosms set up in triplicates using A) river and B) reservoir water.
Fig 4.13: Number of peaks (means and std error) from 16S rRNA gene TRFLP profiles of bacterial communities treated with algae exudates (mixture of exudates from *Chlamydomonas, Chlorella* and *Synedra*) and leaf leachates (mixture of red oak, witch hazel and corn) in experimental microcosms set up in triplicates using A) river and B) reservoir water.
contributes to higher chl $a$ concentration, while in fall, a decrease in algal growth may lead to lower chl $a$ values. Bacterial community structure differed between the river and reservoir at all times but was not related to environmental parameters. However, functional responses did not always differ between the ecosystems. This indicates that differences in the proportion of DOC source may or may not translate to differences in bacterial response.

In spite of the differences in relative concentration of dominant DOC source, overall DOC concentration in river and reservoir sites was mostly similar. The exception to this trend was the site in the reservoir located farthest away from the river (site L3): DOC concentration at this site was higher than that of the river sites in summer. High autochthonous production at L3 in summer (suggested by high chl$a$ concentrations) may have resulted in higher DOC concentrations. However, the lack of difference among the other river and reservoir sites, in spite of differences in color: chl $a$ ratio, suggest that overall DOC concentration may not differ among ecosystems with proportional differences in DOC input. Such decoupling of DOC concentration with source is consistent with Worrall and Burt (2007), but contrary to observations of Westerhoff and Anning (2000), Mullholland (2003), and Wu et al. (2007). For example, Wu et al. (2007) observed that there was a strong linkage between DOC concentration and fluorescent properties of DOC. However such relationships were lacking in the current study.
Bacterial community structure, as determined by redundancy analysis of TRFLP relative peak abundance, was clearly different among the river and reservoir sites on all dates, while within ecosystem variation was also observed. Difference in bacterial community composition in ecosystems with differing source of DOC was also observed in a study by Kritzberg et al. (2006). Although they compared two spatially separated lakes, unlike a reservoir and a lake in the current study, those ecosystems had significant differences in autochthonous production and allochthonous inputs. Even though it can be argued that spatial separation of the two lake ecosystems may have played a role in the difference observed in bacterial community structure so that differences may arise due to limitation in dispersal of bacteria (Yanarell and Triplett, 2005), this may not be the case in the current study. In this study, however, differences in community composition were not related to proportional differences in DOC input. While it is true that the study by Kritzberg et al. (2006) observed links between community structure and variables related to DOC input such as color and chl \(a\), relation to proportions of autochthonous and allochthonous DOC was not discussed in the study. On the other hand, Jones et al. (2009) did observe niche partitioning among bacteria, related to differences in proportion of DOC inputs, unlike the current study.

Temporal differences in community structure were also observed, but were not apparent on all dates and did not necessarily follow trends of change in environmental variables over time. Physicochemical variables like temperature are often linked to changes in community structure (Lindström et al., 2005; Shade et al., 2007). However, in this study, although temperature was higher in the summer (23-June, 27-July and 25-
August) compared to fall (5-October), differences in bacterial communities did not
demonstrate this trend. The relationship of structure to environmental factors does vary
among sites (Boucher et al. 2006, Shade et al. 2008). Although Ghiglione et al. (2005)
suggested a minimum of a two week time interval between sampling periods while
studying temporal differences in bacterial communities, in this case, change in
environmental variables did not relate with structural differences in bacterial
communities even though samples were collected approximately every four weeks.

In spite of the temporal and spatial differences in community structure, contrary
to expectations, no relationship was observed between bacterial community structure and
environmental variables measured, i.e., color: chl a ratio, temperature, color, chl a, DO,
DOC, SRP and nitrate concentrations. Differences in communities occurred in spite of
similarities in DOC, SRP and nitrate concentrations between the river and reservoir.
Additionally, differences in DO and temperature between the ecosystems were not
directly related to differences in community structure. This is not consistent with other
studies that have demonstrated strong correlations of these environmental variables with
bacterial community structure (Crump and Hobbie, 2005; Wever et al., 2005; Rubin and
Leff, 2007). Although the environmental factors considered in this study did not relate
with bacterial community structure, additional factors not measured here, such as
turbidity, conductivity and availability of electron acceptors (Bryant et al., 2012), may
have been more influential in shaping bacterial communities in these ecosystems.
Even under controlled laboratory conditions, different DOC sources did not result in changes in bacterial community structure. In these experiments, leaf leachate represented allochthonous DOC while algae exudate served as a source of autochthonous DOC. The observation was in contrast to experiments performed by Kritzberg et al. (2006) where amendments with algae exudates, leaf leachates and fulvic acids led to differences in bacterial community structure. TRFLP analysis was also used in that study to determine community structure and hence observational differences due to use of different analytical approach is not likely in this case. In terms of bacterial inocula used for the experiment, while Kritzberg et al. (2006) used bacteria from two temperate lakes differing in the dominant carbon source (autochthonous versus allochthonous), bacteria from a river and a reservoir was used in the current study. Hence, it appears that source of bacterial inocula can be a factor in determining differences in community structure with DOC source, as also suggested by Langenheder (2005, 2006).

Bacterial abundance was similar between the river and reservoir sites and showed a positive relation with temperature, SRP, nitrate and DOC concentration while the relation with the color: chl $a$ ratio was negative. The negative relation with color: chl $a$ indicates that with higher proportion of terrestrial DOC input, bacterial abundance decreased. This is in agreement with other studies that have shown that terrestrial inputs include aromatic compounds which are generally not conducive to bacterial growth (Jones et al., 2009). On the other hand, high color: chl $a$ ratio also implies low chl $a$ concentrations and can indicate lesser autochthonous DOC production (Bukaveckas et al., 2011). Autochthonous DOC is considered more labile and hence can be expected to
enhance bacterial growth, as has been observed in other studies (Kritzberg et al., 2006; McCallister et al., 2006; Maki et al., 2010). Low chl $a$ concentration, in that case, can lead to lower bacterial numbers. Thus, although DOC concentration can have a positive influence on bacterial abundance, the source of DOC is important as well. Bacterial abundance was not different among river and reservoir sites even though temperature, SRP concentration and color: chl $a$ ratio were different. Other factors, not taken into account in the study, such as inputs of terrestrial bacteria into the river or attachment of bacteria to the phytoplankton in the reservoir, may have counterbalanced any difference in bacterial counts that may have arisen due to differences in environmental factors measured. Within each ecosystem, however, bacterial abundance followed the opposite pattern of change over time compared to color: chl $a$ and was negatively related to it. This indicates that some of the environmental variables are better predictors of bacterial response within each ecosystem than between the river and reservoir ecosystems.

Bacterial respiration, like abundance, differed among sampling dates but not among sites and was positively related to temperature but not any of the other environmental variables. Such a dependence of bacterial respiration on temperature was expected based on the observations from other studies (Apple et al., 2006; Hall and Cotner, 2007; Sand-Jensen et al., 2007; Lee et al., 2009). However, in this case, reservoir sites had higher temperature than the river sites, at all times, but bacterial respiration rates did not differ. A possible explanation may be that although temperature was higher in the reservoir than the river, other factors like elements involved in respiration, such as Fe and Mn may have been limiting in the reservoir such that not all the available oxygen was
used for bacterial respiration. Also, lack of a relation between respiration and DOC concentration and source was unexpected. DOC concentration or source has been found to be related to respiration rates in several cases (Hanson et al., 2003; Sobek et al., 2006; Lee et al., 2009), unlike the current study. This reinforces the prediction that some other environmental factor, such as those mentioned above, may have been limiting for bacterial respiration. On the other hand, temperature served as a better predictor of bacterial respiration within each ecosystem, as both temperature and bacterial respiration were lowest on 5-October, 2011, compared to the other dates, in the river and reservoir.

Bacterial heterotrophic productivity, unlike abundance and respiration rate, was higher in the reservoir sites than the river on 23-June and 25-August, 2011 and was positively related to temperature, chl a and nitrate concentrations. Although temperature and chl a were also higher in the reservoir than the river on those dates, nitrate concentration was mostly similar among the sites. While the correlation of bacterial productivity with temperature is common (Kirchman et al., 2005; Apple et al., 2006), relationships with nitrate concentrations have also been observed (Kan et al., 2006). In this study, productivity in the reservoir was higher along with temperature on certain dates, but on others, productivity did not demonstrate differences with the river in spite of higher temperatures. This means that, although productivity was related to temperature and nitrate concentration, differences in these variables between the two ecosystems does not necessarily indicate a difference in bacterial heterotrophic productivity. A similar relationship was observed with chl a concentrations as well. Although, in general, a relationship between biomass of primary producers and bacterial heterotrophic
productivity has generally been observed (Castillo et al., 2004; Kritzberg et al., 2005; Kan et al., 2006; Lee et al., 2009), differences in chl \( a \) did not coincide with differences in bacterial productivity among sites in this study. Surprisingly, no relationship was observed between bacterial productivity and DOC concentration or color, as has been found elsewhere (Findlay et al., 2003; Judd et al., 2006; Lennon and Cottingham, 2008). This, combined with the fact that productivity is related to chl \( a \) concentration, suggests that, in the current study, autochthonous carbon source is more important in determining productivity rates within each site, compared to external inputs or overall DOC concentration.

In the experimental microcosms, a difference in bacterial productivity was observed between leaf leachate and algae exudates treatments. For river water, higher DOC concentration at the end of the experiment for algae exudate amended microcosm, compared to leachate amendment, coincided with lower productivity. On the other hand, productivity was higher in algae exudate amended microcosms when water and bacteria from the reservoir was considered, while the final DOC concentration was lower. The results demonstrate that even though algae exudates are usually considered a more labile source of DOC (Grossart and Simon, 1998), the effect of DOC source on bacterial productivity depends more on the source of bacteria rather than the quality of DOC. Bacteria from the river, like other lotic ecosystems, were perhaps more adapted to utilization of allochthonous DOC sources, such as leaf leachates, in spite of their recalcitrant nature (Tank et al., 2010) which stimulated their productivity to a greater extent compared even to more labile autochthonous sources.
Bacterial growth efficiency (BGE) is an estimate of the proportion of assimilated carbon that is used towards bacterial heterotrophic production (del Giorgio and Cole, 1998). BGE did not differ significantly between the river and reservoir sites in spite of a positive relation with color:chl a ratio. BGE was also positively related to color and negatively related to SRP concentration, and like BGE, these environmental parameters were similar between the river and reservoir. A possible reason why BGE did not demonstrate a difference between river and reservoir sites, in spite of differences in color: chl a between the two ecosystems, may have been due to its relationship with color, which was similar between both ecosystems. This is in agreement with other studies which have also demonstrated the importance of terrestrial DOC inputs for bacterial growth (Berggren et al., 2007; Ram et al., 2007). Although prior studies demonstrated that bacterial growth may be positively related to nutrient concentrations (Smith and Prairie, 2004; Berggren et al., 2007), the relation with SRP was negative in this study. The negative relationship of BGE with SRP was reflective of temporal differences in BGE within each ecosystem such that BGE was lower on the dates that SRP was higher and vice versa. Also, color: chl a was higher on 5-October, 2011 and so was BGE, compared to most other sampling dates.

Differences in bacterial community structure did not always follow the same trends as functional aspects. For example, community structure was different between the river and reservoir sites on all dates. This difference was not found in bacterial abundance, respiration or growth efficiency, which were similar between the river and reservoir at all times. Although While Eiler et al. (2003), Kirchman et al. (2004) and
Kritzberg et al. (2006) found a relationship between bacterial community structure and function, under differing concentration or quality of DOC, Van Mooy et al. (2004), observed no such relationship. However, it should be noted that the above mentioned studies that have demonstrated a link between structure and function were carried out under relatively stable laboratory conditions while Van Mooy et al. (2004) observed a lack of structure function relationship, similar to the current study, under variable environmental conditions in the North Pacific.

Under laboratory conditions too, bacterial community structure and function were also not related to each other. Microcosms demonstrated differences in bacterial productivity between leaf leachate and algae exudate treatments but no difference in bacterial community structure. This was in agreement with experiments performed by Langenheder et al. (2005, 2006) where it was observed that bacterial responses related to carbon utilization were better explained by growth medium or bacterial source compared to bacterial community composition.

Overall, a difference was observed between the river and reservoir sites in the dominant source of DOC as determined by the color: chl a ratio. Bacterial community structure was also different between the river and reservoir sites. However, such differences were not reflective of the environmental parameters measured, including those related to DOC source and concentration. On the other hand, bacterial functions, except productivity, were relatively similar between the river and reservoir sites, on all dates. Although certain environmental variables were better predictors of temporal
differences in bacterial function within each ecosystem, they did not explain the patterns of difference between the river and reservoir. Differences in community structure did not translate to functional differences, not only in field conditions, but also under more controlled laboratory conditions as well. This indicates that bacterial communities, differing in structure may adapt to the different environmental conditions to perform similar functions. On the other hand, results from the experimental microcosms indicate that autochthonous and allochthonous sources of DOC can be utilized by similar bacterial communities from a common source resulting in a difference in heterotrophic productivity.
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Heterogeneity of DOC source plays a key role in determining bacterial community structure and function in aquatic ecosystems (Olapade and Leff, 2005; Lennon and Cottrell, 2008; Peter et al., 2011). However, the importance of DOC quality to bacteria, relative to more quantitative aspects (for example, presence or absence of source or relative proportions of inputs), and its influence in linking bacterial community structure and function are not well established because conflicting results have been found in prior studies (Reinthaler et al., 2005; Bell et al., 2009; Frossard et al., 2011; Kritzberg et al., 2006; Murray et al., 2007; Obernosterer et al., 2010). Likewise, in this study, the impacts of DOC varied depending on the particular attributes of the bacterial community that were investigated and DOC quality was the most important factor in determining such differences. Moreover, throughout this research, I examined the effect of DOC on both bacterial community structure (gene diversity and gene abundance) and function (respiration, productivity, denitrification, photosynthetic uptake) and observed that structural and functional responses were not coupled.
In the second chapter of my dissertation, I investigated, whether, in a highly productive lake ecosystem (Lake Erie), presence of a dominant primary producer (*Microcystis*) led to changes in bacterial community structure and function. Particle attached and free-living bacterial communities were studied and compared over time, in the presence and absence of the cyanobacteria. Although community structure differed between the two fractions on each date, with a single exception, similarities were observed between them when compared among different dates. Differences in community structure between the two fractions, on the same date, were observed both in the presence and absence of *Microcystis*. This indicates that structural differences in bacterial communities are not directly influenced by the presence of a dominant primary producer. When attached to particles, however, bacteria were at a functional advantage; generally, bacterial abundance, heterotrophic productivity and uptake of algal exudates were higher in this fraction compared to free-living bacteria. Also, bacterial heterotrophic productivity showed a positive relation with primary productivity. Differences in bacterial community structure, however, were not coupled to functional differences.

In summary, results from chapter 2 indicate that presence or absence of a dominant primary producer may not influence bacterial responses; but when present, these DOC sources can have a positive influence on bacterial functions. While some of the functions such as bacterial heterotrophic productivity may be directly related to primary production, others like bacterial abundance or uptake of photosynthetic exudate are not. In other ecosystems too, the positive correlation between algal biomass or primary production and bacterial heterotrophic productivity has commonly been observed
Bacterial abundance is usually observed to covary with other environmental factors, such as temperature and predator abundance (Le et al., 1994; Coveney and Wetzel, 1995; Gurung and Urabe, 1999, Staroscik and Smith, 2004; Gurung et al., 2010). The lack of relationship between algal variables and uptake of exudates by bacteria was unexpected and may have been a function of bacterial enzymatic activity (Arnosti et al., 2005; Arnosti, 2011). Bacterial community structure also differed based on attachment to particles but it may not be due a selective effect of primary producers on certain communities, since such differences were observed even in their absence. This agrees with some studies that have demonstrated that bacteria can be generalists with respect to particle colonization (Riemann et al., 2000; Ghiglione et al., 2009) as opposed to others that have observed specialization in bacterial communities for colonizing particles in the presence of primary producers (Moeseneder et al., 2001; Fandino et al., 2001; Grossart et al., 2007). The study also demonstrated a lack of structural and functional coupling in bacterial communities. An attempt to relate bacterial structure to function in this study lends new insights to understanding the effect of primary producers on bacterial responses in freshwater lake ecosystems.

Sources of DOC in streams are heterogeneous compared to lakes (Trumbore et al., 1992; Allan and Castillo, 2007) and bacterial responses often seem to relate to such variations (Koetsier III et al., 1997; Olapade and Leff, 2005; Singer et al., 2010; Perryman et al., 2011). These DOC sources include both allochthonous inputs from the riparian zone (Tank et al., 2010; Pollard and Ducklow, 2011) and in-stream
autochthonous production (Risse-Buhl et al., 2011). Bacterial communities are capable of utilizing these varied carbon sources but demonstrate niche partitioning in the process (Olapade and Leff, 2005, Perryman et al., 2011). For example, while some communities can better utilize leaf leachates, others prefer algae exudates (Olapade and Leff, 2005). However, the above mentioned studies have mostly compared bacterial response to broad classes of DOC compounds, while response to heterogeneity within such broad categories needs further investigation.

The effect of molecular heterogeneity within each broad class of DOC, on bacterial responses were investigated in my third chapter, where I treated stream bacteria, grown on artificial substrates, i.e., ceramic tiles (aerobic incubation) and sand (anaerobic incubation), with multiple labile and recalcitrant compounds. Bacterial responses were compared among compounds within each category. Bacteria were also treated with the mixture of compounds from each category (labile or recalcitrant) followed by a comparison of responses between the single and mixed treatments. Chemical diversity within each category of DOC did result in differences in community structure in aerobic bacteria, but only among chemically defined labile treatments. Others, namely, chemically defined recalcitrant compounds, algae exudates and leaf leachate treatments, led to no such differences. For anaerobic bacteria, differences in overall community structure were observed between single and mixed treatments in all cases, and in some cases among single treatments. Denitrifier community structure and denitrification rates differed between single and mixed treatments more often than among single treatments. In fact, mixed labile treatments unexpectedly demonstrated lower
denitrification rates compared to single treatments. Denitrification rates did not co vary with denitrifier structure. Take together this suggests that, even within each broad category of DOC, differences in chemistry can influence bacterial community structure and function. The most dramatic differences were observed between single and mixed DOC sources than among single treatments. Also, structural and functional changes did not always co vary with differences in DOC source, such that similar communities demonstrated differences in functional potential while in other cases different communities were functionally similar.

The influence of mixed DOC treatments on bacterial responses was a noticeable aspect of the study. A study by Wu et al. (2009) found no difference in bacterial community structure between single and mixed treatments with leaf leachates. Although the same was observed in this study when aerobic microcosms were treated with leaf leachates, community structure of anaerobic bacteria differed between the treatments. Since DOC concentration did not decrease significantly at the end of the experiment for the aerobic microcosms, unlike the anaerobic ones, it may be predicted that aerobic bacterial communities may not have been able to uptake DOC from the leachates as efficiently as those from the anaerobic microcosms. Thus, molecular diversity can have a significant influence on bacterial responses, but consideration of a wider variety of bacteria is important.

Denitrification rates also differed between single and mixed treatments using labile compounds with defined chemistry. However, the outcome was contrary to
expectations. Mixture of labile treatments led to lower denitrification rates compared to single compounds, while rates were similar among single treatments. A possible explanation, derived from periodic monitoring of denitrification rates through the incubation period was that, mixtures of compounds support stable rates of denitrification over time while treatment with single labile compounds leads to sudden changes in rates. This in turn may have happened due to competition among bacteria to perform alternative processes that can be carried out in similar conditions, such as dissimilatory reduction of nitrate to ammonium, also referred to as DNRA (Burgin and Hamilton, 2007). It is possible that single labile treatments being less heterogeneous may have been used for one process or the other at a time thereby explaining the sporadic stimulation and high rates of denitrification at the end of incubation. On the other hand, mixtures being more heterogeneous can be used for more than one process at a time, and hence can lead to more stable, but lower rates of denitrification. Thus, mixed treatments can have a more stabilizing effect on ecosystem functions. Future investigations can be carried out to compare rates of different ecosystem processes between such single and mixed treatments to better understand its role in maintaining functional diversity in ecosystems.

Denitrifier structure demonstrated differences not only between the single and mixed labile treatments but also among the single treatments; a pattern not observed in denitrification rates. Also, while the communities treated with glucose and mixture of labile compounds were similar, they demonstrated differences in denitrification rates. In addition, differences were observed in denitrification rates of single and mixed treatments for recalcitrant compounds but the denitrifier community structure remained similar.
Thus, in this study, as in the previous one (chapter 2), community structure was not related to function. A lack of coupling between denitrifier structure and function was also observed in some other studies (Rich and Myrold, 2004; Enwall et al., 2005; Dandie et al., 2008; Song et al., 2010; Attard et al., 2011). On the other hand, Rich et al. (2003), Kjellin et al. (2007), Phillipot et al. (2009) and Peralata et al. (2010) have demonstrated links between structure and function of denitrifiers. The relationship appears to be dependent on the source of bacterial communities because, while Rich et al. (2003) observed linkages in denitrifier structure and functions in forest and meadow soils, Rich and Myrold (2004) found no such relationships in agroecosystems. Hence, differences in molecular heterogeneity of DOC can lead to differences in bacterial structure and function, but whether the two are linked depends on the source of bacteria and the ecosystem studied.

The previous chapters of my dissertation indicated that DOC sources can have an important influence on both lentic and lotic bacterial responses. In my fourth chapter, I addressed the question if bacterial response to DOC in these ecosystems depend on whether the inputs are dominantly allochthonous or autochthonous. For my study, I chose a riverine-lacustrine gradient with differences in dominant DOC inputs. Such differences were determined from color: chlorophyll $a$ (chl $a$) ratio, with color serving as a proximal measure of terrestrial inputs and chl $a$ as an indicator of algal biomass, which in turn is representative of autochthonous DOC production (Jones et al., 2009). Bacterial community structure, respiration, productivity and growth efficiency were compared between a riverine and a lacustrine ecosystem. Also, experimental microcosms were set
up in the laboratory to look at the effect of alternative DOC sources (autochthonous and allochthonous) on similar bacterial communities. Bacterial community structure differed between the two ecosystems at all times, but the differences were not related to environmental variables, including proportions of allochthonous and autochthonous DOC. Bacterial abundance, respiration and growth efficiency were similar between the two ecosystems. Bacterial heterotrophic productivity differed between the riverine and lacustrine ecosystems on a few occasions and was positively related to primary productivity but such relationships helped explain seasonal variations within each ecosystem and not the differences between ecosystems. Also, treatments of similar bacterial communities with leaf leachates and algae exudates did not lead to differences in bacterial structure, although productivity differed. This indicates that, not the proportion of allochthonous or autochthonous DOC input into an ecosystem, but the qualitative differences between the two DOC forms, may be a cause of differences in bacterial function. Furthermore, structure and function appeared to be uncoupled in these ecosystems.

Although differences in proportions of allochthonous and autochthonous inputs in lotic and lentic ecosystems do not determine bacterial responses, a possible influence of terrestrial inputs was observed. For example, differences in proportions of autochthonous and allochthonous DOC did not play a role in explaining structural and functional differences between the riverine and lacustrine bacterial communities. However, color was positively related to BGE and both variables did not differ between the two ecosystems. The positive relationship of bacterial growth with color indicates an
important influence of aromatic terrestrial inputs on bacterial growth in both ecosystems. Such a role played by terrestrial inputs was also observed in other studies (Berggren et al., 2007; Ram et al., 2007; Berggren et al., 2010). A possible reason for such an influence may be the recalcitrant nature of such inputs (Jones et al., 2009) which then makes it a more stable source of carbon, thereby ensuring a homogenous distribution between riverine and lacustrine ecosystems, as opposed to labile autochthonous sources that are likely to be utilized rapidly near their source (Tank et al., 2011). Therefore, bacterial structure and function are not influenced by differences in proportions of autochthonous and allochthonous inputs, but rather, terrestrial inputs can have a positive influence on bacterial growth irrespective of such differences.

In my dissertation, I studied the effect of DOC source and quality on both lentic and lotic bacteria. The results demonstrated that smaller differences in DOC chemistry have a bigger impact on bacterial responses than the general category of the source (allochthonous versus autochthonous; or a dominant autochthonous source). Past studies have often classified lotic and lentic ecosystems as net autotrophic or heterotrophic, based on proportions of autochthonous and allochthonous inputs, and have compared DOC dynamics among ecosystems based on such classifications (Pollard, 2004; Kritzberg et al., 2005). The fourth chapter of my dissertation shows that such generalized categorization may not help in predicting bacterial DOC use in ecosystems and comparing them with others. Rather, predictions related to DOC use may be more accurate if quality of inputs is considered, as demonstrated by the third chapter. Allochthonous and autochthonous inputs may differ in quality among ecosystems (Sell and
Overbeck, 1992; Tank et al., 2010) and such differences deserve more emphasis, rather than differences in their proportions. In addition, chapter 2 demonstrates that presence or absence of a dominant DOC source does not influence bacterial responses and instead, bacterial functions are enhanced only after associations with the source are made. As a result, carbon budgets, involving bacterial use of DOC should not just rely on the presence or absence of sources, but should take into account the compositional heterogeneity of such sources and the differential response of bacteria to resource diversity.

The studies from my dissertation also show that while DOC has an influence on both bacterial community structure and functions, the two were generally not linked. In other words, different bacterial communities have been observed to perform similar functions (functional redundancy). Functional redundancy can be beneficial for ecosystems because loss of certain taxa due to sudden changes in environmental conditions will not mean a loss of functions (Bowen et al., 2011). In spite of the fact that functional redundancy was observed in all the studies that I conducted, as described in chapters 2, 3 and 4, these results did not agree with the outcomes of other studies investigating bacterial structure-function linkages (Rich et al., 2003; Kjellin et al., 2007; Phillipot et al., 2009; Peralata et al., 2010). This makes it imperative to look at relationships between bacterial structure and function in each case when predicting the effects of DOC sources on ecosystem stability, especially in aquatic ecosystems where bacteria act as key mobilizers of DOC (Tranvik, 1992; Findlay, 2010).
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