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Phytotoxic effects of terrestrial dissolved organic matter on a freshwater cyanobacteria and green algae species is affected by plant source and DOM chemical composition

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HIGHLIGHTS

- Spectroscopic and elemental measures of DOM differed depending upon the plant source.
- These same measures were used to predict DOM phytotoxicity to a cyanobacteria.
- Angiosperm DOM was more phytotoxic to a cyanobacteria, and less so to a green algae.
- Divergence in algal sensitivity shows vegetation may affect algal assemblages.

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ABSTRACT

Here we link plant source phylogeny to its chemical characteristics and determine parameters useful for predicting DOM phytotoxicity towards algal monocultures. We found that DOM characterised using UV–visible spectroscopic indices and elemental analysis is useful for distinguishing DOM plant sources. Specifically, combined values of absorbance at 440 nm and coefficients for the spectral slope ratio, were used to distinguish between gymnosperm-leached DOM and that from angiosperms. In our bioassays, DOM leached from 4 g leaf L^{-1} resulted in over 40% inhibition of photosynthetic yield for the cyanobacterium, *Cylindrospermopsis raciborskii*, for eight of the nine plants tested. Significant variables for predicting inhibition of yield were DOM exposure time and plant source, or using an alternate model, exposure time and spectroscopic and elemental measures. Our study proposes spectroscopic indices which can estimate a plant source's contribution to aquatic DOM, may provide insights into ecological outcomes, such as phytotoxicity to algae. The cyanobacterium (*C. raciborskii)* was more sensitive to DOM than a green algae (*Monoraphidium spp.*), as identified in a subsequent dose-response experiment with five different DOM plant sources. Low level additions of angiosperm derived-DOM (i.e. 0.5 g L^{-1}) were slight phytotoxic to *Monoraphidium spp.* causing 30% inhibition of yield, while *C. raciborskii* was not affected. Higher DOM additions (i.e. 2 g L^{-1}) caused 100% inhibition of yield for *C. raciborskii*, while *Monoraphidium spp.* inhibition remained under 30%. The divergence in algal sensitivity to DOM indicates that in aquatic systems, DOM derived from catchment vegetation has the potential to affect algal assemblages.

1. Introduction

Dissolved organic matter (DOM) is involved in major biogeochemical processes of the carbon cycle, and modulates the structure and function of freshwater ecosystems (Jansson et al., 2007; Steinberg et al., 2006). For example, DOM can cause changes in algal communities (Jones, 1992; Scharnweber et al., 2014). DOM can both inhibit and stimulate algal growth depending on its chemical composition. Small molecular mass DOM may function as a nutrient source to algae, providing an accessible source of carbon, nitrogen and/or phosphorus (Berman and Chava, 1999; Fiedler et al., 2015; Qin et al., 2015). Conversely, some DOM

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forms have been found to inhibit algal growth. Mechanisms include adsorption of inorganic nutrients which limits their availability for uptake (Murray et al., 2010); reduced light availability for photosynthesis (Bährs and Steinberg, 2012); or acting as a natural biocide (Park et al., 2009a). These DOM-algal interactions are highly unpredictable as DOM chemistry is dependent on the source in which it is derived.

Compound-specific bioassays have identified that phenols and quinones may act as a natural biocides towards algae (Bährs et al., 2013; Nakai et al., 2001). These compounds arise naturally from the breakdown of lignin in leaves (Abrosca et al., 2006; Nakai et al., 2001), and these phenolic substances are commonly used for tracing DOM sources (e.g. Bährs et al., 2012; Pillinger et al., 1994). This is attributed to their relatively inert characteristics, contributing to environmental persistence, and their distinct composition enabling source identification (Jex et al., 2014). New rapid and cost effective methods using UV–visible spectroscopy have recently been applied to characterize DOM. These includes an estimation of DOM lignin phenol content and aromaticity that have been employed to estimate DOM source and quality in marine water samples (Cuss and Guéguen, 2013; Helms et al., 2008; Qin et al., 2015). However, the application of spectroscopic methods for identifying sources of lignin compounds and associated phenolic characteristics in freshwater systems remains unclear.

The chemical composition of leaf litter is species-specific, thus soil-derived DOM characteristics are related to forest community composition (Gartzia-Bengoetxea et al., 2016; Moingt et al., 2016). These between-species differences in soil DOM chemistry are useful for predicting nutrient turnover rates (Wieder et al., 2008), toxicological effects on soil microbes (e.g. microbial activity, Gartzia-Bengoetxea et al., 2016), and can also be used to trace the source of soil DOM (Jex et al., 2014). However in comparison to terrestrial environments, few studies in aquatic systems have investigated if plant phylogeny affects the chemical composition of DOM. Exceptions include recent work tracing the origin of DOM. These studies have identified broad differences between DOM originating from angiosperms versus gymnosperms, and woody versus non-woody parts of plants (Cuss and Guéguen, 2013; Maie et al., 2006; Seki et al., 2014). However it is uncertain whether such methods are able to distinguish between individual plant species and, in addition, predict phytotoxicity of plant leachates. Use of spectroscopic indices for the characterisation of DOM may provide a useful method to link the DOM plant source to its effect in an ecosystem measure, which in our case study, is toxicity to algae.

The main stress response often measured in toxicological studies to estimate phytotoxicity of DOM to algae is reduced photosynthesis (Bährs et al., 2012; Gattullo et al., 2012). This is because DOM is proposed to inhibit algal photosynthesis by quenching electrons or binding to the bio-quinones in PSII, to prevent electron transfer (reviewed in Prokhotskaya and Steinberg, 2007). Active DOM compounds appear to be phytotoxic to prokaryotic algae (i.e. cyanobacteria), but harmless to eukaryotic algae (i.e. green algae) at the same concentration (Bährs et al., 2013; Park et al., 2009b; Prokhotskaya and Steinberg, 2007). Research is required to establish under what conditions DOM is a nutrient source or an algaecide.

Our study investigates the effect of plant source on DOM characteristics, and DOM chemistry on inhibition of algal photosynthesis, to determine if it is viable to predict DOM phytotoxicity. Further, this study compares the differing sensitivities of a prokaryotic algae and a eukaryotic algal species to DOM.

2. Methods

Dissolved organic matter was leached from leaves, characterized and then supplied to algal monocultures in two bioassay experiments. The first experiment examined the effects of DOM plant source phylogeny and chemical composition of DOM on the photosynthetic yield of the cyanobacterium, *C. raciborskii*, over 15 d. The second experiment compared the sensitivity of two different algal species, using a prokaryotic algae (cyanobacterium, *C. raciborskii*) versus a eukaryotic algae (a green algae, *Monoraphidium spp.*), over a concentration gradient of DOM exposure loads, for five different DOM plant sources.

2.1. Terrestrial DOM collection and preparation

Terrestrial leaf material was collected from vegetation commonly found along rivers and creeks in southeast Queensland, Australia. The plants selected consisted of seven distinct families that represented a phylogenetically distinct suite of terrestrial leaf types. These consisted of three non-woody angiosperms, Poaceae (*Digitaria didactyla, Leersia hexandra*), Cyperaceae (*Schoenoplectus litoralis*) and Asparagaceae (*Lomandra hystrix*), two woody-angiosperms, Myrtaceae, and Casuarinaceae (*Casurina cunninghamiana, Allocasurina littoralis*), and two woody-gymnosperms, Araucariaceae (*Araucaria cunninghamii, Araucaria bidwillii*) and Pinaceae (*Pinus radiate, Pinus ponderosa*). Due to the highly distinct characteristics of oils released from within the Myrtaceae family, this group was further explored at the genus level; *Melaleuca spp., (M. linariifolia, M. quinquenervia), Eucalyptus spp., (E. tereticornis, E. grandis)* and *Lophostemon spp., (L. suaveolens, L. confertus).*

Green leaves from plants were collected in the field seven days prior to use. Leaves were taken from three different individual plants of each species, and stored in individual paper bags. In Expt. 1, the leaf material from two plant species of each family was combined to make one treatment ready for subsequent leaching (Table 1). However, for two of the treatments, viz. Cyperaceae and Asparagaceae, only one plant species was used, as a second similar species was not found in abundance within any of the plant collection areas. In Expt. 2, leaf material from each plant species was leached separately, to increase reproducibility of experiment design and results. On arrival at the laboratory, leaf material was rinsed (2–5 s) under running distilled water and patted dry. Large leaves were cut into 5–7 cm pieces, and oven dried at 50 °C for 48 h (Martin and Ridge, 1999). Leaf material was soaked in deionized water (autoclaved, 18.1 MΩ cm, Arium® 611 UV system, Sartorius) at a ratio 1: 20 w/v for 24 h. This was conducted in dark conditions preventing growth of photosynthetic microbes and photochemical modification of the DOM (e.g. production of ammonium or reactive oxygen species, Stedmon et al., 2007; Wang et al., 2000).

Leachates were separated from the solid material by successive filtration; first through a 0.20 μm bottle top filter (Filtropur BT 50, Sarsted Inc, USA) under light vacuum, and then through a 0.22 μm membrane filter syringe under sterile conditions (Minisart, Sartorius). Leachates were diluted with autoclaved deionized water (1:40 w/v), and the pH adjusted to 6, by the addition of sterile 1 N sodium hydroxide (NaOH). DOM leached directly from the leaf source were used, rather than river water DOM, as the latter consists of DOM at various stages of diagenesis and can be from mixed origins (Cuss and Guéguen, 2013). The leachates were used within 2 h of filtering to avoid any effects of storage on DOM chemical properties (Cuss and Guéguen, 2013). In this study, sterilising agents, such as sodium azide, were not used as they can cause chemical changes in DOM that will interfere its spectroscopic properties due to fluorescence quenching (Cottrell et al., 2013; Cuss and Guéguen, 2013; Pisani et al., 2011). In addition, collected leachates were used in bioassays, therefore the use of agents such as sodium azide was unadvisable as it can impair photosynthetic performance (Nultsch et al., 1983) or cause minor growth