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Application of a microalgal slurry to soil stimulates heterotrophic activity and promotes bacterial growth

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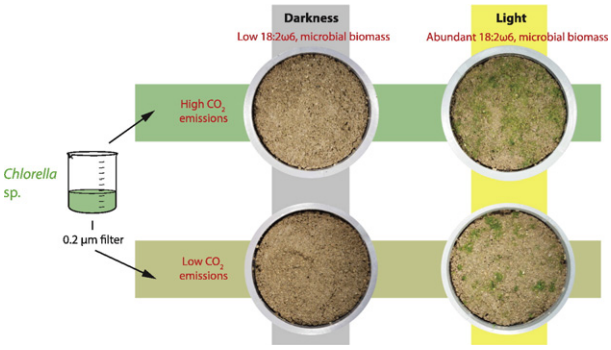
HIGHLIGHTS

- The microalgal suspension stimulated soil CO_2 production.
- The photosynthetic microalgal suspension accelerated formation of a soil biofilm.
- The photosynthetic suspension reduced carbon mineralization by an average of 25%.

GRAPHICAL ABSTRACT



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ABSTRACT

Active microalgae biomass from wastewater treatment may be given added value as a biofertilizer, but little is known about how this may affect soil nutrient dynamics and biology. If the goal is to recycle waste nutrients and matter, live algae applied in a liquid slurry to soil may add both organic carbon and nutrients while providing other benefits such as biological carbon fixation. However, the potential persistence of unicellular green algae after such an application is not known, nor the influence of their photosynthetic activity on soil organic carbon - the aim of the present study was to probe these basic questions. In a controlled laboratory microcosm experiment, suspensions of *Chlorella* sp. microalga culture and sterile filtrates were applied to an agricultural soil and incubated for 42 days, whereas the effect of darkness was also tested to understand the importance of photosynthetic activity of the algae. Autotrophic microorganism development was 3.5 times higher in treatments with algae application as measured by chlorophyll pigment concentration. Against expectations that increased photosynthetic activity would decrease the CO_2-C flux, the algal suspension with a photoperiod significantly increased soil respiration compared to culture filtrates without algal cells, with accumulated quantities of 1.8 and 0.7 g CO_2-C m^{-2}, respectively. Also, phospholipid fatty acid (PLFA) analyses showed that the suspension accelerated the development of a stable community of eukaryotic and prokaryotic microorganisms in the soil surface, whereas bacterial PLFA biomarkers were significantly associated with eukaryote biomarkers on the study level.

1. Introduction

Bioremediation of wastewaters with microalgae, which began in the 1950s, is now a widely researched field with numerous historical

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applications (Oswald, 2003). The harvest and utilization of algal biomass produced in waste treatment is of increasing interest since microalgae may produce bioactive substances such as phytohormones or accumulate elements of interest (Mallick, 2002; Stirk and van Staden, 2010). Other uses, specifically as fertilizer in sustainable agriculture, has also been a topic of research (Michalak et al., 2016); however this has been often limited to seaweeds or the application of dried and/or deceased biomass. The study of living, unicellular, photosynthetic biomass in biofertilizer applications has been somewhat limited to N_2-fixing (diazotrophic) cyanobacteria in rice paddies in particular. Applications in aerobic soils are much more limited in number, many of these probing cyanobacterial plant growth promotion products (Nain et al., 2010; Rana et al., 2012). Aerobic soil applications with living algae present an entirely different scenario; microalgae are specifically adapted to aquatic environments, requiring continuous hydration for maintenance of cellular structure, nutrient acquisition, and gas diffusion. As such, in soil environments, the proliferation of microalgae is strongly conditioned by humidity, and temporal desiccation will occur in arid and semi-arid environments where the benefits of soil organic matter may be greatest. Despite these obvious constraints, microalgae, alone or as a component of biological soil crusts (BSC) play roles in soil nutrient cycling and water fluxes (Maestre et al., 2011). Microalgae play a vital role in fixing carbon and nitrogen, stabilizing the soil, and altering the hydrological properties (e.g. water retention) of crust-covered soils in arid and semi-arid environments (Evans and Johansen, 1999; Belnap and Lange, 2003). Therefore, activities of microalgae improving soil functions and properties might be enhanced and exploited through applications of biofertilizers containing living algal biomass.

Apart from functions improving soil health, soil applications of microalgae may also serve for mitigation or sequestration of atmospheric carbon dioxide. Though autotrophic microorganisms are not generally thought to have a key role in CO_2 fixation and sequestration in soils, global net carbon uptake of cryptogamic covers from the atmosphere amounts to ~3.9 Pg yr^{-1}, which is on a scale similar to the global annual carbon release due to biomass burning and fossil-fuel combustion, respectively (Elbert et al., 2012; Yuan et al., 2012).

Implemented in photobioreactors, microalgae can metabolize and accumulate residual nutrients from agro-industrial waste (Morales-Amaral et al., 2015). We have hypothesized that when algal biomass is applied in the field as a biofertilizer, this will effectively increase soil nutrients and contribute to increased CO_2 fixation thereafter. This potential to increase soil organic matter content and fix additional atmospheric C into cropland soils must be tested. There is currently very little insight about how soil biology will respond to the application of an algal suspension biofertilizer in a general sense. For instance, it is not known whether algae applied to the soil in relatively high densities (while cell sizes and numbers vary, dry matter harvesting from photobioreactors can be from 0.5 to 1.3 g L^{-1}) will quickly decease and decompose or instead create a stable community in the soil. With the large-scale addition of (alga) autotrophs to soil, trophic interactions may occur with microorganisms responsible for decomposition and any concomitant influence(s) on the composition or density of the dominant soil microorganisms responsible for short-term decomposition in agricultural soils. Since mutualistic interactions between prokaryotic and eukaryotic cells in biofilms improves their resistance to harsh environmental conditions, phototrophic soil biofilms may present an improved strategy for nutrient management, and at present investigations on the application of biofilms to benefit agriculture are scarce (Swarnalakshmi et al., 2013).

Chlorella (Chlorophyta) is a genus of non-motile green alga whose species can possess a mixotrophic metabolism (Ogawa and Aiba, 1981); due to the capacity for heterotrophy along with high growth rates and ease of cultivation, it has become a model organism for photobioreactor and wastewater treatment technologies (Oswald, 2003; Wang et al., 2012). In a soil microcosm experiment, we applied a *Chlorella* sp. suspension to test key hypotheses; first, we expected that this would increase soil carbon and nitrogen (total C and N contents). Second, that increased autotrophic soil algae populations would cause reduced soil respiration. Third, we also hypothesized that the addition of algae would stimulate exo-enzymatic activities, particularly those associated with the decomposition of cellulose and simple sugars (β-glucosidase, α-glucosidase) found in algal exudates and from the turnover of algal cells. In order to test these hypotheses, the study was designed to simulate application of algae to an agricultural soil in a laboratory setting, with measurements of parameters of interest at different small-scale soil depths in order to better understand the localized effects of the augmented autotrophic microbial biomass component.

2. Materials and methods

2.1. Soil

The soil samples were taken from the Ap horizon (0–20 cm) of an irrigated field located in Losar de la Vera (Cáceres, Spain) N 40° 1′ 52.85″ W 5° 36′ 49.29″. In this field, soil was sampled in four evenly-spaced locations distributed within 1 ha, mixed together, and sieved to <2 mm. The soil is classified as *Dystric regosol* (*Rd*) according to FAO (IUSS, 2006), with a sandy-loam texture (sand 76.5%, loam 11.0%, clay 12.5%). Soil physical and chemical properties are (mean values of three replicates): bulk density 1.3 g cm^{-3}; soil water-holding capacity (WHC) 51.4% v/v; pH (water 1:2.5 w/v) 6.4; electrical conductivity (water 1:5 w/v, 25 °C) 0.32 dS m^{-1}; soil organic matter 1.2%; total nitrogen 1.1 g kg^{-1}, and available phosphorus (Bray and Kurtz, 1945) 39 mg kg^{-1}.

2.2. Soil microcosms

Addition of the microalgal suspension to the agricultural test soil was tested with incubation in both light and dark conditions (SAL and SAD, respectively) in order to separate the effect of photosynthesis from other potential effects on the CO_2 flux. Also, since the algae culture contains nutrients and this can influence soil metabolism, the effect of applying only a sterile filtrate (0.2 μm) of the culture medium was also tested in both light (SL) and darkness (SD). Dark treatments were achieved by placing an opaque but ventilated box into the same growth chamber, and it was confirmed with measurements that light did not actually enter this box and that the temperature was the same inside and out. Finally, to isolate the CO_2 fixation potential of algae with no soil, an equivalent amount of algal culture used in the soil treatments was applied to inert fiberglass filters (A). With this experimental design, the objective was to explicitly test the effects of light versus darkness and the inclusion versus exclusion of living algae biomass, and for this reason other factors were not included (for instance addition of water only, since it was not an objective to test the effects of BG11 nutrients). These treatments are summarized in Table 1. Four replicates of each

Table 1
Study treatment codes and descriptions: the application of unfiltered algae suspensions (treatment acronym with "A", otherwise as sterile filtrates), treatments to soil (with "S", otherwise to inert filters), incubated with a photoperiod (with "L") or in darkness (with "D").

Treatment code	Description
AL	*Chlorella* sp. suspension on fiberglass filter incubated with 16:8 photoperiod
SAD	Soil and *Chlorella* sp. suspension incubated in darkness
SAL	Soil and *Chlorella* sp. suspension incubated with 16:8 photoperiod
SD	Soil and growth media filtrate (<0.2 μm) incubated in darkness
SL	Soil and growth media filtrate (<0.2 μm) incubated with 16:8 photoperiod