



Effects of biosolids on biodiesel crop yield and belowground communities



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ARTICLE INFO

Article history:

Received 7 November 2013

Received in revised form 1 March 2014

Accepted 31 March 2014

Available online 4 May 2014

Keywords:

Biosolids

Biodiesel crops

Automated ribosomal intergenic spacer analysis (ARISA)

Earthworms

Heavy metals

Seed yield

ABSTRACT

Biofuels are an attractive alternative for fossil fuels and demand is growing rapidly, however the environmental impacts of biofuel production must be minimized. Replacing conventional fertilizers with biosolids, processed solids from municipal wastewater treatment plants, has the benefit of utilizing a waste stream and avoiding some of the environmental impacts associated with conventional fertilizers, but the impacts of heavy metals in biosolids must be assessed. In a pot trial, we grew two oilseed crop species, *Brassica napus* and *Camelina sativa*, in soil amended with two levels biosolids and soil amended with urea. Seed yield and oil content were compared between soil treatments, and effects on soil chemistry, activity of microfauna, and bacterial and fungal community structure were quantified. We also measured the impacts of biosolids addition on the growth, survival and tissue chemistry of earthworms. Seed yield of plants grown in biosolids was comparable to or greater than that of plants fertilized with urea. Biosolids addition increased soil concentrations of plant nutrients, but also heavy metals (e.g. arsenic, lead, chromium and nickel). Microfaunal activity, as well as soil microbial community structure, was impacted by both fertilizer type and oilseed plant species. Earthworm biomass was enhanced by addition of biosolids though earthworms exposed to biosolids had elevated levels of copper. Our results suggest biosolids could effectively fertilize these oilseed crops and may enhance soil health, but impacts of heavy metals should be considered.

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1. Introduction

Liquid fuels produced from renewable biological materials are an attractive alternative to fossil fuels with the potential to reduce greenhouse gas emissions and increase energy independence. Many governments have established biofuel production targets, and as a result global demand is expected to double from levels experienced in years 2005–2007 by 2017 (Bringezu et al., 2009).

However, for biofuels to be a sustainable alternative to fossil fuels the energetic costs of production and environmental impacts must be minimized (Pickett et al., 2008; Zah et al., 2009).

The transportation sector contributes significantly to global warming with 10% of this radiative forcing attributed to CO₂ emissions from road transport alone (Fuglestedt et al., 2008), and there is a global shift in demand from gasoline to diesel fuel (OPEC, 2012; Conti et al., 2013). Half of the transport fuel consumed in New Zealand is diesel (New Zealand Ministry of Economic Development, 2011) making biodiesel a potentially important way to reduce the country's greenhouse emissions. Oilseed crops, grown as break crops in 3–4 year rotation with cereals, are a part of the New Zealand biodiesel industry and may be used to produce high quality biodiesel following the transesterification of plant oils. Previous research has identified *Brassica napus* (or canola) and *Camelina sativa* as biodiesel crops that are particularly adaptable

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to New Zealand growing conditions (McKenzie et al., 2011; Fasi et al., 2012).

Biofuel crops face many of the same environmental challenges as other annual crops. These crops often require nitrogen fertilizers to compensate for low nitrogen use efficiency and high nitrogen demand, which can result in nitrate (NO_3^-) contamination of waterways, soil acidification, and significant fluxes of nitrous oxide (N_2O), a potent greenhouse gas, from soil. Furthermore, nitrogen fertilizers are energetically expensive to produce (i.e. 0.9–1.8 kg carbon equivalents per kg fertilizer as estimated by Lal, 2004). These factors can decrease the potential benefits of replacing fossil fuels with biofuels (Crutzen et al., 2008; Robertson et al., 2011).

Biosolids are processed solids from municipal wastewater treatment plants, and are applied to agricultural land in many countries with or as an alternative to conventional fertilization regimes, with the added benefit of using a waste stream. Nitrogen release from biosolids is slower than from chemical fertilizers (Claassen and Carey, 2007), potentially improving their efficiency to supply plant available N over an extended time period and decreasing the amount of N reaching waterways. They are also a source of other nutrients, including phosphorous, but can contain heavy metals with concentrations depending on the municipal source. Municipal biosolids is a waste stream, often destined for landfill in New Zealand due to limited beneficial reuse options. Furthermore, there are public and stakeholder concerns about application to land for food production placing further constraint on its use. The concept of using biosolids to grow biofuel crops eliminates some of these concerns and thus provides a beneficial reuse option for a significant societal waste stream. Furthermore, it is an elegant solution for whole life-cycle product management, where biodiesel glycerol, a bi-product of biofuel production can be used to generate methane via anaerobic digestion at municipal sewerage treatment plants (Weber et al., 2012). This biogas can then be used to dry wet biosolids (70–75% moisture) into a dry product (5–10% moisture) suitable for spreading as a fertiliser on biofuel crops.

While agricultural biosolids application limits have been designed to minimize the potential of NO_3^- leaching, the impact of heavy metals and other contaminants on soil biota must also be considered as the abundance, diversity and activity of soil organisms are intrinsically linked to soil health. For example, earthworms and other soil fauna play an integral role in decomposition and nutrient mineralization from organic matter (e.g. Verhoef and Brussaard, 1990; Steinberg et al., 1997; Bardgett and Chan, 1999; Cole et al., 2000), hyphae of soil fungi help to maintain soil structure (reviewed by Tisdall, 1991), and soils with more diverse microbial communities may be more resistant to pathogen invasion (van Elsas et al., 2012). There are several mechanisms by which biosolids could negatively impact soil biota and other organisms; (1) changes in soil communities, which may alter soil function, (2) direct mortality or fitness impairment of sensitive soil species and (3) heavy metals may enter the food chain through the crops and/or geophagous organisms and subsequently bioaccumulate in higher trophic levels.

The aim of this work was to evaluate how using biosolids from municipal waste, as an alternative to chemical fertilizer, would impact seed yield of biofuel crops, soil chemistry and belowground communities. In a pot trial we grew *B. napus* and *C. sativa* in soil amended with biosolids, urea and in control conditions. Changes in soil chemistry, microfauna activity, and microbial community structure due to soil treatments and plant species were quantified at the end of a five month growing period. In addition, we measured how long-term exposure to biosolids impacted earthworm growth, survival and heavy metal accumulation.

2. Materials and methods

2.1. Experimental setup

Field soil was collected from Ashley Dene Farm located near Lincoln University in Canterbury, New Zealand ($43^\circ 39' \text{S}$, $172^\circ 19' \text{E}$). The soil type is a stony Lismore silt loam and the paddock was previously used for dryland pasture. Soil was mixed with sterilized pumice grade 1–3 mm in a 3:1 ratio to improve aeration and drainage. Equal volumes of the soil mixture were added to 80 plastic pots (10 L) for ten replicate pots per combination of soil treatment (control, conventional fertilizer, low biosolids and high biosolids) and oilseed plant species (*B. napus* and *C. sativa*). Only the soil pumice mixture was added to the control soil treatment. The conventional fertilizer treatment was a single addition of 200 kg N/ha as urea when most plants had reached green bud stage (approximately 5 weeks after sowing), as is common for canola production. The high and low biosolids treatments were 60 g and 30 g of dry biosolids (total N equivalents of 316 kg N/ha and 158 kg N/ha). These values were chosen to test for effects of biosolids addition both below and above the guideline of 200 kg N/ha/yr provided by the New Zealand Water & Waste Association (2003). Biosolids were added to the pots prior to sowing and mixed into the soil to a depth of approximately 5 cm to approximate typical practice of incorporating biosolids into soil. Biosolids used in this experiment were generated from municipal wastewater at Christchurch City Council's Bromley Wastewater Treatment Plant and met the NZWWA Grade a pathogen standard and Grade b maximum contaminant levels (NZWWA, 2003).

Twelve seeds of *B. napus* (spring cultivar 'Ability') or *C. sativa* accession no. 4164 (Emcy Enterprises, Australia) were initially sown in each pot and plants were subsequently thinned allowing two plants per pot to grow to harvest. Plants were grown over the summer in a shadehouse with treatment combinations assigned randomly to positions within the shadehouse. Pots were watered daily and weeded regularly.

2.2. Seed yield, chemistry and plant biomass

Plants were harvested by clipping at the soil surface approximately five months after sowing when they had begun to senesce. Pods were cut from plants and seeds were separated by hand. Seed yield (adjusted for seed moisture), aboveground dry matter, and number of pods were measured for individual plants. Harvest index was calculated as the contribution of the dry seed to the total aboveground dry matter weight and expressed as a percentage. Seeds from the two plants per pot were pooled and thousand seed weight was calculated from the weight of four sets of 200 seeds when possible. In cases where seed yield was insufficient for this approach, thousand seed weight was calculated from the weight and number of all seeds harvested from the pot.

To determine oil content of the seed three 2.0 g subsamples of dry seed, pooled by plant species and soil treatment, were ground with 0.75 g celite to aid in grinding (two subsamples were analyzed for *C. sativa* grown in control soil due to insufficient seed). Total lipids were extracted by Soxhlet extraction with hexane using a BUCHI Soxhlet Extraction Unit E-816HE (BUCHI Labortechnik, Flawil, Switzerland). Subsamples of the resulting oil and seed residue were extracted with nitric acid and hydrogen peroxide and digested in a CEM Mars Xpress Microwave Digestor (CEM Corporation, Matthews, North Carolina, USA). Concentrations of 19 elements in the digested samples, including 10 heavy metals, were determined with a Varian 720 ICP-OES (Varian Australia Pty Ltd, Melbourne, Australia).

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