Evaluating Microdictyon umbilicatum bloom biomass as an agricultural compost conditioner for native and commercial plants

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Evaluating Microdictyon umbilicatum bloom biomass as an agricultural compost conditioner for native and commercial plants

Abstract
To date findings demonstrate that the Microdictyon umbilicatum bloom biomass can be effectively composted with terrestrial greenwaste to provide a soil conditioner with a useful macronutrient and trace element profile, and with no evidence of metal contamination of concern. As expected with an unwashed marine algal source, the sodium concentrations were elevated and dosing of % seaweed biomass in compost should be controlled accordingly. Native plant growth responses to Microdictyon umbilicatum enhanced compost were positive up to 5% compost content in a 1:1 mix with potting mix (total 2.5% seaweed biomass), with increased growth rates for both species; saltbush (Rhagodia candoleana) and Coastal Banksia (Banksia integrifolia). Salt bush maintained enhanced growth rates for the 10% and 20% compost additions as well, but showed most benefit at 5% as an increase in leaf abundance as well. Banksia did not respond as well to higher seaweed concentration composts.

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Evaluating *Microdictyon umbilicatum* bloom biomass as an agricultural compost conditioner for native and commercial plants.
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Executive Summary

This study supports the use of the macroalgal (seaweed) biomass from a *Microdictyon umbilicatum* bloom in Jervis Bay, NSW, as a safe and beneficial organic additive to compost and soils. The resulting compost was categorised as an Australian standard Grade A soil conditioner or mulch with no evidence of metal or other contamination of concern. This grade allows for unrestricted use on home gardens, public spaces, urban landscaping, agriculture, forestry, soil and silo rehabilitation, landfill disposal or surface land disposal. The findings demonstrate that the *Microdictyon umbilicatum* bloom biomass can be effectively composted with terrestrial green waste to provide a soil conditioner with a useful macronutrient and trace element profile. In addition it is of benefit to the growth and stress tolerance of native species and edible crops respectively. As expected with an unwashed marine algal source, the sodium concentrations were elevated at high doses and 5% volume of seaweed biomass content in compost, assuming an addition ratio to soil or potting mix of 1:1, is recommended.

Native plant growth responses to *Microdictyon umbilicatum* enhanced compost were significantly increased by 157% and 73% for Saltbush (*Rhagodia candoleana*) and Coastal Banksia (*Banksia integrifolia*) respectively. In addition the numbers of leaves for Salt Bush were significantly greater with 5% seaweed biomass volume compared to controls. Both plant species maintained good growth rates at higher seaweed compost additions up to 20%, but there was no significant benefit. This optimal range of seaweed biomass in compost is a bell curve response that might reflect plant sensitivity to sodium salts at higher seaweed content. Prewashing the salt from the seaweed biomass may extend the range of beneficial seaweed content, but this would require further testing for confirmation.

Radish (*Raphanus sativus*) harvestable biomass was on average greater, but not significantly so, for the 5% compost treatment. Tissue analysis of radish biomass revealed a significant increase of carbon and silicon in the plant tissue for all macroalgal compost treatments. In contrast, sulphur, potassium and magnesium were all reduced in plant biomass. There was no indication of a risk of increased metal uptake with seaweed biomass; in contrast reduced metal uptake was indicated.

It is recommended that future bloom biomass of *M. umbilicatum* seaweed can be used to condition soils at concentrations up to a total of 2.5% or in compost at 5% for both native and edible plants species. As an organic additive in soils, this provides for both beneficial plant health and an effective and valuable use of a marine resource.
Introduction

In 2010, a major green tide bloom event of \textit{Microdictyon umbilicatum} occurred in the popular coastal, tourist destination of Jervis Bay, NSW. The biomass from the bloom was extensive and washed up onto beaches throughout the Bay in large quantities that remained for over 1 year. There were serious consequences for public amenity and management challenges about how to address the issue. Considering that the cause of the bloom was not established and there is little evidence for \textit{Microdictyon} sp. responding rapidly to sewage or other high nutrient sources, the options for management of future blooms included investigating the removal of biomass and resource development as compost for application on land plants.

Macroalgal blooms, or ‘green-tide’ events, are becoming increasingly widespread and have been reported all over the world (Morand and Briand 1996; Jorgensen et al., 2010). Green tides are usually linked to eutrophication or upwelling events (Liu et al., 2009) as many green macroalgal species respond opportunistically to high nitrogen levels. Blooming genera include \textit{Ulva}, \textit{Chaetomorpha}, and \textit{Cladophora} (Maze et al., 1993; Vallini.G. et al., 1993; Hiraoka et al., 2004; Liu et al. 2009; Jorgensen et al. 2010) which have been investigated as potential sources of organic biomass for composting (Maze et al. 1993; Cuomo et al., 1995; Wosnitza and Barrantes 2006). Research has shown that biomass from these genera can provide for both safe and beneficial application on land crops. Benefits include boosted yields (Gandhiyappan and Perumal 2001), salinity tolerance (Nabti et al., 2009), and disease resistance (Paulert et al., 2009).

\textit{Microdictyon} is a less commonly reported genera in macroalgal blooms despite evidence of \textit{Microdictyon} spp. blooms from remote areas (Vroom and Timmers 2009) and elsewhere in calmer water environments similar to Jervis Bay (Wallis Lake) (Great Lakes Council 2005). This green, coenocytic (non-cellular) genus has strong vegetative and opportunistic reproduction and growth strategies (Kim et al., 2002), however it is less well understood what key triggers might cause \textit{M. umbilicatum} blooms. Unlike for \textit{Ulva} spp., which respond rapidly to ammonium supply (Vandermeulen Gordin 1990) through either eutrophication, natural upwelling or run off events (Jorgensen et al. 2009), nutrient preference studies have not been undertaken for \textit{M. umbilicatum} in literature searches. We found only 2 published articles on its biology in peer reviewed phycological journals (Kim et al., 2002; Liao et al., 2003). What is known however is that this genus can bloom sporadically in near pristine, marine protected environments without human impacts, and with little consequence for the health of the ecosystem function in the long term (Vroom Timmers 2009). As \textit{Microdictyon} spp. are less common in macroalgal bloom events it has not been previously investigated for safe use or potential benefits as a compost for land vegetation.
Because the bloom event in Jervis bay created massive accumulations of macroalgae it began to decompose, produced unpleasant odours for locals and tourists and impacted recreational activities along the shoreline (i.e. boating, surf clubs races, joggers etc). As it is not desirable to increase the load of organic waste to landfill, Shoalhaven City Council proposed to begin the first study evaluating Microdictyon umbilicatum bloom biomass as an agricultural compost conditioner for native and commercial plants. The bloom event described above demonstrated the necessity of establishing appropriate use strategies for the biomass for potential future events. This is particularly important for many blooms events where the actual cause of the algae proliferation is unknown and remediation of coastal nutrient sources in not an option.

The application of algae or algal extracts to benefit plant growth has been demonstrated throughout the literature and has been recognised in the scientific arena since at least as far back as WWII and for use even earlier (see review by Craigie (2010)). The function of algae applied to plants can be either through delivery of a diverse array of essential nutritional and trace elements, or through inducing disease resistance (Figure 1). Some benefits of improved nutrition or disease resistance include improved seed germination, increased root growth and higher yields, earlier harvests, increased frost resistance, reduced incidence of insect attack, or longer shelf-life of produce (Abetz.P. 1980; Blunden.G. 1991; Greger et al., 2007; Eyras et al., 2008; Rayirath et al., 2009; Quilty and Cattle 2011). In addition, several studies have shown that improved physical characteristics of the soil also contribute to improved plant health through increased porosity and an increase in water holding capacity (Haslam and Hopkins 1996; Eyras et al., 1998). In addition seaweeds also contain plant growth promoting hormones including cytokinins, auxins, and gibberellins (Quilty and Cattle 2011).

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**Nutritional benefits**

- Nitrogen
- Phosphorous
- Potassium
- trace elements

**Disease resistance**

- sulphated polysaccharides

**Endocrine effects**

- cytokinins
- auxins
- gibberellins

**Soil conditioning**

- water holding capacity
- beneficial soil biota

---

![Figure 1. Four pathways in which macroalgae can contribute to plant health.](image)

The benefits and risks posed by using seaweed as an organic additive in composts or soils vary according to the quality of the coastal water source (i.e. no toxic heavy metal pollutants) and the species of seaweed used as the chemical composition of seaweeds varies. Some are benign, others beneficial and some may be toxic to herbivores (e.g. Caulerpa sp. (Amade and Lemee 1998)) which may or may not be suitable for composting. In addition the application may be suitable for a limited range of crops. For example a head to head comparison of some red and brown seaweed species in
composts as well as compost without seaweed demonstrated superior yields from red seaweeds, but also greater transfer of cadmium to some crop plants (Greger et al. 2007). Greger et al (2007) therefore recommended not to apply the red macroalgal biomass to edible crops, but highlighted its potential suitability for non-food crop cultivation. However the variability of source and effect and the metal content in seaweeds depending on species and environment suggests that further assessment of macroalgal composts of various species and applications on diverse plants is required. As algae grow rapidly in response to nutrients and are effective “sequenchers” of metals and radioactive substances, metal as well as radio-active toxicity of seaweed biomass on plants, the environment and humans through plant ingestion is possible (Greger et al. 2007; Schmidt 2011). The benefits or risks of using Microdictyon species is not known and must be established before recommendations on its use can be made.

The current project objectives were to assess the use of M. umbilicatum as a compost conditioner by:

1) Evaluating the composting process of adding different quantities of macroalgal bloom biomass;
2) Determining whether applications of M. umbilicatum enhanced compost has any effect on a selection of native species used in local, coastal revegetation projects; and
3) Determining the effect of bloom biomass enhanced composts on edible plant seedlings.
Methods

Collection of *Microdictyon umbilicatum* bloom biomass from Jervis Bay

*M. umbilicatum* was collected from Currambene Creek (Woollamia Boat Ramp) in Jervis Bay from the 4\(^{th}\) – 8\(^{th}\) March 2011 under the collection permit F95 269-6.3 (I&I NSW Fisheries). *M. umbilicatum* was being washed seaward with the tide along Currambene Creek and becoming lodged amongst seagrass beds of *Zostera capricorni* at a depth of approximately 0.5m (m) and 10m from the shoreline. Three hundred and fifty litres (L) of *M. umbilicatum* bloom biomass was collected via sweep nets and included a mixture of algae species dominated by *M. umbilicatum* (approx. 70%) but included the marine plant *Zostera capricorni*, phaeophytes including *Padina* sp., *Sargassum* sp., *Cystophora* sp., *Dictyota* sp., chlorophytes including *Colpomenia* sp., *Caulerpa* cf. remotifolia, *Caulerpa cactoides*, *Caulerpa filiformis* and rhodophytes including *Gracilaria* sp., *Rhodoglossum* sp., *Asparagopsis* sp. (Figure 2). This assemblage represents a macroalgal and marine plant mix which would be washed up on the beach in a bloom event with varying proportions. Subsequent bloom biomass showed increased dominance of *Rhodoglossum* sp. but this biomass was not tested. The algae were transported to the composting site and composts were established within 1 week of collection.

*Figure 2. Collected Microdictyon umbilicatum* biomass (top) and other seaweed species mixed in the *Microdictyon umbilicatum* biomass (Bottom).*
Compost conditioning with *Microdictyon umbilicatum* bloom biomass

*M. umbilicatum* was added to partially composted, shredded green waste (mulch) and fresh green waste (grass) at three compositions: 0% (control), 5%, 10% and 20%. The fresh grass was added to aid the breakdown process as the mulch was sourced from a large windrow which already had been through an initial composting phase. The harvested *M. umbilicatum* was very dense, which had to be compensated for in establishing an equivalent volume compared to the mulch and fresh grass. The seaweed was spread out onto cardboard and using a garden fork was tossed to break up clods and to add air into the material (more comparable to the aerated mulch and fresh grass) (Figure 3). One cubic metre (1m$^3$) of each compost treatment was prepared. Table 1 provides the relative volumes for each composition.

![Figure 3. Seaweed was spread out on cardboard (left) and tossed using a garden fork to approach a comparable density to that of the green waste source. Each layer in the compost was watered using a microbe inoculant at a ratio of 10:1 (right).](image)

Table 1. Volume and layers of seaweed, fresh grass and mulch in the four different compositions (including control.)

<table>
<thead>
<tr>
<th></th>
<th>Layers</th>
<th>5% Seaweed composition</th>
<th>10% Seaweed Composition</th>
<th>20% Seaweed Composition</th>
<th>Layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Control</td>
<td>0 Layers</td>
<td>0.05m$^3$ Seaweed</td>
<td>0.1m$^3$ Seaweed</td>
<td>0.2m$^3$ seaweed</td>
<td>10 Layers</td>
</tr>
<tr>
<td>0.3m$^3$ fresh grass</td>
<td>10 Layers</td>
<td>0.25m$^3$ fresh grass</td>
<td>0.2m$^3$ fresh grass</td>
<td>0.2m$^3$ fresh grass</td>
<td>10 Layers</td>
</tr>
<tr>
<td>0.7m$^3$ mulch</td>
<td>11 Layers</td>
<td>0.7m$^3$ mulch hl</td>
<td>0.7m$^3$ mulch</td>
<td>0.6m$^3$ mulch</td>
<td>11 Layers</td>
</tr>
<tr>
<td>T = 1m$^3$</td>
<td>T = 21 Layers</td>
<td>T = 1m$^3$</td>
<td>T = 1m$^3$</td>
<td>T = 1m$^3$</td>
<td>T = 31 Layers</td>
</tr>
</tbody>
</table>
Each treatment compost bay was layered with seaweed, fresh grass and mulch for a total of 31 layers (Figure 4). The control bay was layered alternately with grass and mulch only (21 layers in total). To further enhance the breakdown and to improve the quality of the compost, it was spray watered with a microbial inoculant (liquid bokashi lactobacillus brew) in water at a ratio of 10:1 during the initial compost set-up phase (2 litres for each composition heap) and each time during the fortnightly turning of the compost. Thirty litres of fresh water was also added to each bay during the set-up process. The fortnightly turning and inoculation was undertaken to achieve a faster breakdown process within 8 weeks. The final turning included watering with a 20:1 water/molasses mix to assist the breakdown through one more rise in temperature.

With the remaining seaweed, two fermentations were prepared to speed and improve the decomposition process. First equal amounts of seaweed and sawdust bokashi were prepared and blended together and liquid bokashi was mixed through (Figure 5). The final mixture was place in a large sealed bag and the air removed. This is an anaerobic preparation. The second preparation used three litres of seaweed in a 20 litre bucket with 6 litres of water.
At the first turn two weeks after establishment, material around the edge of the compost piles showed the layering of the mulch, grass and seaweed. The rest of the material showed good initial breakdown with only small pockets of grass and clumps of seaweed (Figure 6). During the turning a conscious effort was made to blend outside materials to the inside and top and bottom together to achieve a more homogenous mass. After each bay was emptied of its compost, it was blended and placed back into the same bay. Water and microbe inoculant was sprayed as in the initial construction of the compost piles.
Temperature and Oxygen in the core of each compost heap was monitored throughout the breakdown period (~8 weeks) on a weekly basis. The compost heaps reached 50°C after 1 week, which is an appropriate temperature for break down of the organic material through microbial growth. The oxygen readings were high due to the openness of the material in the heaps due to the large particle size and number of woodchips in the compost (essentially compost mulch). The dryness of the compost heaps was likely due to the high amount of wood chip (i.e. large particles) in the heaps resulting in more air space. In normal compost the particle sizes are lower, giving a denser pile and less air space. This is not an ideal compost heap for maximum nutritional profile, but it is sufficient to represent real green waste resources and to digest the seaweed as a waste management strategy. After 8 weeks the compost fever declined, indicating a reduction in organic material and final maturing (Figure 7).

![Figure 7. Initial temperature of green waste and M. umbilicatum compost mix over 6 week duration of maturation.](image)

In order to achieve a homogeneous potting mix grade product, each of the 4 compost blends was sieved through a 16mm screen (Figure 8). This removed the large twigs and woodchips from the humus and smaller particles. Anecdotal observations suggested that the 20% seaweed blend contained more humus, had a deeper colour and was the most moistest. The control had the least amount of humus and was the most dry.
Compost chemical and nutrient analysis

The 4 different compost treatments, the pure potting mix and the pure *M. umbilicatum* mix were analysed for macro- and micronutrient profiles and for the pathogens *Escherichia Coli* and *Salmonella* at the NATA accredited Environmental Analysis Laboratory (EAL in NSW). Total ash and acid digest was used to determine phosphorus, calcium, magnesium, potassium, sodium, iron, manganese, zinc, Copper, molybdenum, cobalt and boron. Samples were digested on a hotblock digestor using Aqua Regia acid and read on an APHA 3120 ICP-MS (Inductive Coupled Plasma-Mass Spectrometer). The methods used include US EPA Microwave Digestion Methods SW-3015, SW-3051, and SW-3052. Total Nitrogen, Sulphur and Carbon were determined using a LECO CNS2000 infrared gas analyser.

The 20% compost mix was tested to establish safe use and classification against the Soil Conditioner and Fine Mulch Australian Standard AS4454 (US EPA 2000; US EPA 2001; Standards Australia 2003) at the Sydney Environmental and Soil Laboratory (SESL). This included levels of metals, pesticides, physical particles and toxicity.
Application of *M. umbilicatum* biomass to native and commercial plant seedlings

The three completed compost treatments of *Microdictyon umbilicatum* enriched compost (5%, 10%, 20%) and one control compost with no *M. umbilicatum* added (0%), were diluted into a 1:1 potting mix. These compost enriched potting mix treatments were used to re-pot 5 replicates of each of two native revegetation species and for germination of 8 radish seeds (for each treatment) from which replicates of 5 random seedlings were selected (total of 20 seedlings per species).

The native seedling species Saltbush (Rhagodia candoleana) and Coastal Banksia (Banksia integrifolia) were selected to test the compost treatments on native species used in local revegetation programs. Both native species were provided by the Milton Rural Land Care nursery from revegetation stock. *R. candoleana* plants had been propagated from cuttings and were tube stock size, while *B. integrifolia* tube stock had been propagated from collected seeds and were also of tube stock size. These native seedlings were established in controlled environmental conditions in a shade house, and then maintained in natural sunlit conditions until the experimental treatments commenced. The compost treatments were applied to the randomly selected native seedlings by re-potting five of each species into separate 6 inch diameter pots with one of the four treatments. Plants were maintained in greenhouse and natural sunlight conditions and equal watering as required between April and August 2011.

The edible crop (Radish - *Raphanus sativus*) was selected from commercial seed stock to test compost treatment effects on germination, growth rates and tissue composition of edible plants. A total of 8 *R. sativus* seeds were germinated in each of 4 seedling trays using the four treatments (Figure 9). After two weeks five radish seedlings from each of the four treatments were selected at random and replanted in individual 4 inch diameter pots for growth rate monitoring, and an additional 3 seedlings were maintained for desiccation stress tests.

![Figure 9. Seedling germination of *Raphanus sativus* in 0%, 5%, 10% and 20% *M. umbilicatum* compost in a 1:1 mix with potting mix.](image-url)
Plant response monitoring

Native species were monitored for 14 weeks and a number of morphometric measurements were recorded on 8 occasions during that time to compare the trajectories of plant responses across the treatments. Measurements included plant height (mm) and the number of leaves. All plants were watered on an as need basis in response to soil moisture which varied according to weather conditions.

The relative growth of the edible *R. sativus* plants across the treatments was established by measuring biomass after seedlings were maintained for 8 weeks to harvestable size. The plant biomass was extracted from the soil, cleaned and dried at 60°C for 48 hours to a constant weight, and leaf, radish bulb and total dry biomass were recorded. Dried leaves from 3 replicates in each radish treatment and dried bulbs from 2 replicates in each treatment were ground to a powder using an IKA A11 Mill and sent for macro- and micronutrient analysis at a NATA accredited laboratory (EAL) using the same methods used above for the compost samples.

After 8 weeks of monitoring, 3 randomly selected, replicate soil samples from the control group and the 5% treatment of *R. sativus* plants were tested for soil biology at Soil Food Web International. This included the determination of levels of bacteria, fungi, protozoa, nematodes, and mycorrhizal colonization.

Finally a desiccation stress test was undertaken (i.e. no watering) on three additional *R. sativus* plants from each treatment to determine whether the seaweed compost imparts any protections against stress. After 3 weeks, response to stress across the three treatments plus the control plants was measured using a Photosynthesis Yield Analyser MINIPAM. This MIMPAM applies pulse-modulated measuring light for selective detection of chlorophyll fluorescence yield. This yield is used to follow changes in plants brought about by exposure to stress.

**Statistical Analysis**

All univariate data was analysed in the SPSS statistical package according the relevant experimental design using a one-way analysis of variance for each species where n = 5. For soil biological data, PRIMER was used to analyse the changes to multivariate assemblages of soil biota. Data was analysed in both transformed (square root, 4th root and presence/absence data) as well as untransformed formats to determine the relative changes to composition as well as abundance. SIMPER analysis was used to identify the species that contributed most to assemblage change. Further univariate analysis of variance was undertaken to test for species specific change across treatments.
Results

Compost Quality and Safety outcomes

The 20% *M. umbilicatum* bloom biomass was successfully composted with the shredded green waste and produced a dark brown, high moisture and organic rich soil conditioner and fine mulch according to Australian Standards (Table 2). The water content for the 5% compost treatment, at an average of 51%, was slightly higher than the control treatment at an average of 45%. The algae treated compost therefore has greater structure and a greater ability to hold more oxygen and water.

Table 2. Characteristics of 20% *M. umbilicatum* and green waste mixed compost according to Australian Standards AS 4454: Soil Conditioner & Fine Mulch Analysis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unit</th>
<th>Requirement</th>
<th>Result</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:1.5) in water</td>
<td></td>
<td>5.0 - 7.5</td>
<td>7.1</td>
<td>Neutral</td>
</tr>
<tr>
<td>Electrical Conductivity (1:1.5)</td>
<td>dS/m</td>
<td>No limit</td>
<td>3.84</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (P) - Soluble</td>
<td>mg/L</td>
<td>&lt; 0.1</td>
<td>0.11</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Phosphorus (P) - Total</td>
<td>%</td>
<td>&lt; 0.1</td>
<td>Acceptable</td>
<td></td>
</tr>
<tr>
<td>Ammonium-N (NH₄)</td>
<td>mg/L</td>
<td>&lt; 200</td>
<td>2.7</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Nitrate-N (NO₃)</td>
<td>mg/L</td>
<td>≤ 10</td>
<td>0.02</td>
<td>Low</td>
</tr>
<tr>
<td>Plant Available N (NH₄ + NO₃)</td>
<td>mg/L</td>
<td>&gt; 200</td>
<td>&lt; 5.0</td>
<td>Low</td>
</tr>
<tr>
<td>Nitrogen (N) - Total</td>
<td>%</td>
<td>≥ 0.6</td>
<td>0.88</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Organic Matter (Loss On Ignition)</td>
<td>%</td>
<td>≥ 25</td>
<td>43.4</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>mg/kg</td>
<td>&lt; 2000</td>
<td>14.1</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Wettability</td>
<td>mm</td>
<td>&lt; 7</td>
<td>0.07</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Toxicity Index</td>
<td>mm</td>
<td>≥ 60</td>
<td>50</td>
<td>Acceptable for pasteurized</td>
</tr>
<tr>
<td>Particle Size Grading</td>
<td>%</td>
<td>-</td>
<td>99.43</td>
<td>Soil conditioner</td>
</tr>
<tr>
<td>&lt; 16mm dia.</td>
<td>%</td>
<td>-</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>≥ 16mm dia.</td>
<td>%</td>
<td>-</td>
<td>Soil conditioner &gt; 20% and &lt; 70% for pasteurized</td>
<td></td>
</tr>
<tr>
<td>Total CaCO₃ Equivalent (if pH &gt; 7.5)</td>
<td>%</td>
<td>CaCO₃</td>
<td>50</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Moisture content</td>
<td>%</td>
<td>&gt; 25</td>
<td>50</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Glass, metal, rigid plastics &gt; 2mm dia.</td>
<td>%</td>
<td>≤ 0.5</td>
<td>0.0</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Light plastic</td>
<td>%</td>
<td>≤ 0.05</td>
<td>0.0</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Stones &amp; lumps of clay</td>
<td>%</td>
<td>&lt; 5</td>
<td>1.9</td>
<td>Acceptable</td>
</tr>
</tbody>
</table>

The pure *M. umbilicatum* biomass was naturally high in calcium, potassium, magnesium (Figure 10) and sodium (6.7 ppm). Upon dilution with green waste and potting mix the calcium, potassium and magnesium were reduced to acceptable but less than desirable levels. Sulphur and sodium were reduced to suitable levels for the 5% treatment but sodium levels were still high in the 10 and 20% treatments. These levels reflect the addition of 10 and 20%, unwashed seaweed biomass, and consequently a lower proportion of bloom biomass or washed biomass would be desirable from this point of view. *Escherichia coli* counts were elevated but this is normal for the young age of the
compost, and Salmonella was absent. A maturation time of up to 9 months is recommended for the reduction of *E. coli*.

For macronutrients, the phosphorous was very low in the bloom biomass and subsequently in all treatments. The nitrogen content was suitable in the original bloom biomass but decreased substantially upon addition of green waste. Consequently the C:N ratios were high in all treatments due to dilution with a cellulosic heavy woodchip content, and it may be desirable to use a more nitrogen rich source of green waste for an optimal compost profile. A longer incubation time would also lead to increased nitrogen content.

Boron and molybdenum were trace elements that appeared to be provided by seaweed biomass in the treatments (Appendix 1), while the rest of the trace elements were at similar or higher levels in the other organic components of the compost (woodchips, green waste or potting mix). Levels of boron have a maximum range in soil mixtures but the elevated levels of between 24-32mg.kg were well below the allowable 200mg/kg (Australian Standards AS 4454).
Figure 10. Macro nutrient profiles (% dry weights) (a,b) and ratios (c) of the pure *M. umbilicatum* bloom mass (100%), pure green waste compost (C), the three treatment composts with 20%, 10% and 5% *M. umbilicatum*, and typical compost profiles (T) according to Environment Analysis Labs (EAL).
Plant Growth Responses

Native Species

Both native species, *Rhagodia candoleana* and *Banksia integrifolia*, responded positively to the treatments of 5% *M. umbilicatum* compost mixed 1:1 with potting mix (effectively 2.5% *M. umbilicatum* biomass content) (Figure 11). After 3 months of growth in the 5% treatment, *R. candoleana* and *B. integrifolia* showed a 157% and 73% faster specific growth rates respectively, compared to the control group (F=8.9, p=0.001 and F=4.7, p=0.015). The 10% and 20% treatments were not different to the control group suggesting that the salinity might have been too high. This was anecdotally indicated with some delamination and “burn” marks on the leaves of *B. integrifolia* in the 20% treatment. The number of leaves was also significantly greater by 100% for *R. candoleana* (F=8.26, p=0.002) in the 5% treatment (Figure 12). Although there wasn’t statistical significance, there was a clear trend of an increased number of leaves for *B. integrifolia* (F=2.2, p=0.13) in 5%.

![Graph](image1.png)

Figure 11. Specific growth rates of *B. integrifolia* and *R. candoleana* over four weeks using 5%, 10% and 20% *M. umbilicatum* in the compost mix. (n=5).

![Graph](image2.png)

Figure 12. The number of leaves counted on individuals of *R. candoleana* (left) and *B. integrifolia* (right) over 3 months in each of the treatments; control and with 5%, 10% and 20% *M. umbilicatum* compost mix. (n=5).
*Radish (Raphanus sativus)*

Analogous to the native species, *R. sativus* plants responded with a positive growth trend in the 5% compost treatment however this was not statistically significant (F=1.01, p =0.41) (Figure 13). The larger total biomass for the 5% treatment was contributed to both by higher bulb and leaf biomass compared to all other treatments and again the 10% and 20% treatments did not tend to differ from the control group.

*Figure 13. Dry weight biomass of *R. sativus* tissue, separated as bulb (dark shading) and leaf tissue (light shading) biomass (S.E. bars for total biomass shown).*
Plant tissue analysis

Plant tissue macro- and micronutrients in the bulb and leaves of *R. sativus* differed significantly to the leaf tissue content (ANOSIM Global $R=0.411$, $p=0.002$) and was due, as expected, to a higher nitrogen and mineral content in the leaves (Figure 14). Therefore, comparisons between treatments for tissue composition were done separately for the root and leaf biomass.

![Figure 14](image1.png)

Figure 14. The tissue compositional differences between leaf and radish bulb tissue composition for all samples.

Sodium also increased significantly with treatment in the leaf tissue (Figure 15), which is to be expected considering the bloom biomass was not washed and we observed high sodium content in the seaweed mix (Figure 9). These elevated levels of sodium were not present in the most dilute 5% algae compost treatment ($=2.5\%$ algal content), reflecting similar levels to that in the control.

![Figure 15](image2.png)

Figure 15. Percentage sodium in dried leaf and radish bulb tissue ($n=3$ and $n=2$ respectively)
Plant tissue K, S and Mg decreased in treatments compared to the control (Table 3), despite the pure bloom biomass containing relatively high amounts of these three elements. This could either be due to relatively high amounts of these elements in the terrestrial sourced green waste or woodchip biomass, or a reduced uptake from the soil due to other factors. In contrast there were significant increases in silica (15% increase in 5% treatment) and carbon tissue content (between 2-5% across all treatments) (Table 3 and Figure 16). As most of the silica was provided through the green waste and/or woodchip components of the compost mixes, this finding was interpreted as an increased uptake of silica, rather than provision through compost components, by plants treated with macroalgal biomass. Similarly, the carbon content was relatively low in the macroalgal biomass and therefore the increased carbon content in treatments was interpreted as either increased CO₂ production in the soil with macroalgae, or as an increased uptake from the atmosphere by plants with seaweed treatments.

Table 3. Macro and trace element variables tested across the compost treatments(5%T, 10%T, 20%T) for radish leaves and root bulbs (n = 3 and n = 2 respectively).

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Effect on tissue composition</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N %</td>
<td></td>
<td>0.282</td>
<td>1.618</td>
<td>0.237</td>
</tr>
<tr>
<td>P %</td>
<td></td>
<td>0.012</td>
<td>1.906</td>
<td>0.183</td>
</tr>
<tr>
<td>K %</td>
<td>↓ leaf &amp; root</td>
<td>1.358</td>
<td>3.577</td>
<td>0.047*</td>
</tr>
<tr>
<td>S %</td>
<td>↓ leaf</td>
<td>0.356</td>
<td>6.106</td>
<td>0.009*</td>
</tr>
<tr>
<td>C %</td>
<td>↑ leaf &amp; root</td>
<td>2.806</td>
<td>15.098</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ca %</td>
<td></td>
<td>0.235</td>
<td>2.283</td>
<td>0.131</td>
</tr>
<tr>
<td>Mg %</td>
<td>↓ leaf</td>
<td>0.035</td>
<td>3.637</td>
<td>0.045*</td>
</tr>
<tr>
<td>Na %</td>
<td>↑ leaf &amp; root 20%T</td>
<td>0.396</td>
<td>5.578</td>
<td>0.012*</td>
</tr>
<tr>
<td>Cu mg/kg</td>
<td></td>
<td>5.281</td>
<td>1.587</td>
<td>0.244</td>
</tr>
<tr>
<td>Zn mg/kg</td>
<td></td>
<td>112.268</td>
<td>.915</td>
<td>0.463</td>
</tr>
<tr>
<td>Mn mg/kg</td>
<td></td>
<td>157.583</td>
<td>.515</td>
<td>0.679</td>
</tr>
<tr>
<td>Fe mg/kg</td>
<td></td>
<td>2483.205</td>
<td>.312</td>
<td>0.817</td>
</tr>
<tr>
<td>B mg/kg</td>
<td></td>
<td>191.265</td>
<td>.987</td>
<td>0.431</td>
</tr>
<tr>
<td>Mo mg/kg</td>
<td></td>
<td>.728</td>
<td>.873</td>
<td>0.482</td>
</tr>
<tr>
<td>Co mg/kg</td>
<td></td>
<td>.000</td>
<td>.219</td>
<td>0.882</td>
</tr>
<tr>
<td>Si mg/kg</td>
<td>↑ leaf &amp; root 5% T</td>
<td>2124.353</td>
<td>3.343</td>
<td>0.050*</td>
</tr>
</tbody>
</table>
Figure 16. Percentage carbon in dried leaf and radish bulb tissue (n=3 and n=2 respectively).

Plant Soil Biology

The soil biology results demonstrate that the compost had matured sufficiently and contain healthy levels of algae, bacteria and protozoa. The total biomass and numbers of all groups, except for amoebae, were above the expected range for healthy radish soil for both the control and 5% treatments. Total and categorized biomass results can be seen in Table 4. The soil biological analysis revealed a reasonable community of nematodes (soil grazers, feeders and predators) with a total of 12 species (Table 5). Although there was some indication of differences between the diversity of soil nematodes, this was not significant and quite variable (ANOSIM global R = 0.07, p = 0.3).

Table 4. The biomass and number of diverse microbial groups and nematodes in the soil matrix of radish plants from the control and 5% treatment with macroalgal biomass.

<table>
<thead>
<tr>
<th></th>
<th>Active Bacteria (ug/g)</th>
<th>Total Bacteria (ug/g)</th>
<th>Active Fungi (ug/g)</th>
<th>Total Fungi (ug/g)</th>
<th>Total microbe biomass</th>
<th>Flagellates (#/g)</th>
<th>Amoebae (#/g)</th>
<th>Ciliates (#/g)</th>
<th>Nematode (#/g)</th>
<th>Total abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94.5</td>
<td>930</td>
<td>4.99</td>
<td>973</td>
<td>2002.49</td>
<td>3095</td>
<td>618</td>
<td>102</td>
<td>23.3</td>
<td>3838.3</td>
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<tr>
<td></td>
<td>86</td>
<td>1703</td>
<td>0.94</td>
<td>1232</td>
<td>3021.94</td>
<td>3232</td>
<td>1340</td>
<td>107</td>
<td>25.5</td>
<td>4704.5</td>
</tr>
<tr>
<td></td>
<td>122</td>
<td>1113</td>
<td>8.78</td>
<td>1252</td>
<td>2495.78</td>
<td>12548</td>
<td>1003</td>
<td>61</td>
<td>41.9</td>
<td>13653.9</td>
</tr>
<tr>
<td>5% Treatment</td>
<td>94.5</td>
<td>968</td>
<td>1.74</td>
<td>1056</td>
<td>2120.24</td>
<td>5996</td>
<td>2996</td>
<td>125</td>
<td>36</td>
<td>9153</td>
</tr>
<tr>
<td></td>
<td>109</td>
<td>978</td>
<td>4.81</td>
<td>822</td>
<td>1913.81</td>
<td>9816</td>
<td>7858</td>
<td>726</td>
<td>106</td>
<td>18506</td>
</tr>
<tr>
<td></td>
<td>66.6</td>
<td>973</td>
<td>6.76</td>
<td>1012</td>
<td>2058.36</td>
<td>965</td>
<td>965</td>
<td>121</td>
<td>19.9</td>
<td>2070.9</td>
</tr>
</tbody>
</table>
Stress Tests

Plants under non-stress conditions had high photosynthetic response yields that did not differ between treatments. Under stress conditions, the yield response was reduced significantly ($F = 6.127, p = 0.021$) only for the control, indicating that all treatments with seaweed biomass were less susceptible to stress from desiccation with increasing concentration of macroalgal content in the soil (Figure 17). Yield readings of the control plants were reduced under stress by an average of 2.6% to an average yield of 839 Fv/Fm (maximum PSII photochemical efficiency).

![Figure 17. Photosynthetic yield (Fv/Fm) of R.sativus plants after 3 weeks of drought dress in Control, 5%, 10% and 20% M.umbiculatum treatments.](image-url)
Discussion

These findings establish that *Microdictyon umbilicatum* bloom biomass can be effectively composted with terrestrial green waste to provide a soil conditioner with a useful macronutrient, mineral and trace element profile. There was no evidence of metal, pesticide or pathogen contamination of concern. A 5% blend with terrestrial green waste can provide for good compost which when blended with potting mix (or soil equivalent) at a ratio of 1:1 is classified as a Grade A soil conditioner or mulch to Australian Standards. It is unrestricted in its use from home gardens, public spaces, urban landscaping, agriculture, forestry, soil and silo rehabilitation, landscape or surface disposal. As expected with an unwashed marine algal source, the sodium concentrations were elevated for some treatments and dosing of a percentage of biomass in compost should be controlled accordingly if the biomass is not washed prior to use. Carbon and nitrogen levels in the compost were quite low and this can be improved with the type of green waste used for blending with nitrogen rich waste and cellulosic materials to provide for an adequate plant food.

Higher than normal counts of *E. Coli* were detected at the start of cultivation trials after 8 weeks of composting incubation, and this was reflective of a less mature compost. Due to time restraints in this study such comparisons were not possible and inoculants were used to try and boost the maturation process, but it is recommended that longer composting periods will benefit the quality of the compost. Longer maturation times for composting should be adhered to in real applications, and there was no concern for other pathogens. Other studies that provide evidence of beneficial effects of compost on crops have allowed for a longer periods of compost maturation, up to 20 months (Eyras et al. 2008), which will also provide for increased nutrient availability for plants.

All native species examined (*B.integrifolia, R.candolleana*) responded positively to the 5% seaweed compost treatment showing higher Specific Growth Rates (SGR) and higher leaf numbers for *R.candolleana*. A larger biomass was recorded for the edible radish (*R.sativus*) across all treatments and in particular for the 5% treatments, however this was not significant at 8 weeks of growth. Still, these findings suggest that a 5% *M.umbiculatum* mix is the most beneficial proportion for optimal growth rates for both the native revegetation species and the edible crop examined. Numerous studies have revealed a large range of beneficial effects of seaweed conditioner including early seed germination, improved crop performance and yield, elevated resistance to stressors, increased root growth, reduced incidence of insect attack, or longer shelf-life of produce (Abetz.P. 1980; Blunden.G. 1991; Greger et al. 2007; Eyras et al. 2008; Rayirath et al. 2009; Quilty and Cattle 2011). Although many components of seaweed extracts and their modes of action remain unknown, it is thought that a host of interactions of synergistic effects provide benefits for plants (Fornes et al; 2002 and Vernieri et al; 2005). The biostimulatory potential of many plant-growth stimulating proteins, macro-
and micro-element nutrients, amino acids, vitamins, auxins etc., can lead to enhanced growth and crop yield in many plants upon application of macroalgae (Crouch and Van Staden, 1993; Crouch et al. 1992). However, the potential of these factors to stimulate plant growth is still being discovered and their mode of action is largely unknown (Khan et al, 2009).

The addition of a higher percentage of seaweed (10% and 20%) into the compost did not cause adverse effects on the plants but it did not result in measurable growth benefits either. A bell curve type response to the dosing of seaweeds such as that demonstrated here is supported throughout the research literature. For example, Finnie and Van Staden (1985) found that high concentrations of kelp extract applied to tomato plants inhibited root growth but resulted in stimulatory effects at lower concentrations of addition. Temple and Bomke (1988) also found that application of 120t/ha of fresh kelp on bean crops reduced yields, emergence and flowering, yet at 60 t ha-1 yields were increased. They determined, via greenhouse experiments, that these reduced yields were due to soluble salts and we suggest that the elevated sodium levels in radish tissue in this study indicate that elevated salt content was the cause of a decline in beneficial effects of the 10% and 20% seaweed composts. Washing of seaweed biomass may reduce this effect but that remains to be demonstrated.

The trace and nutrient composition of the R. sativus biomass did not indicate any risk of increase in harmful metal uptake in plants grown in the seaweed treatments. In contrast, the seaweed treatments indicated a potential reduction of metal uptake in the case of magnesium. The bioavailability of magnesium in rats has been shown to be reduced in the presence of seaweed carbohydrates which can bind the metals (Kikunaga et al., 1999) and this may have influenced the trend of reduced metal uptake observed in this study.

Further, this study demonstrated that the application of macroalgal biomass provided not only for increased growth, but significantly increased carbon content throughout the plant tissue (R.sativus). This increase in carbon was not related to carbon content in the original compost treatments as this did not differ, but it potentially reflects increased uptake of atmospheric carbon or an increased breakdown (respiration) of organic carbon in the soil over time. There are many factors that can influence carbon content in plants and these include soil carbon pools, microbial turnover, fungal transport, nutrient availability, soil temperature, and soil moisture (McDowell et al., 2004).

Increased stress tolerance of plants in seaweed treatments was demonstrated here and is consistent with other studies (Rayirath et al. 2009). Increased stress tolerance may be due to the increased water holding capacity of soils with the highly charged sulphated polysaccharide molecules from
seaweed, or through other metabolic pathways in the plant itself. Despite this lack of complete understanding, the findings here support the notion that in drought prone regions of Australia, application of macroalgal biomass to vegetation can be beneficial.

Based on the results from this study it is recommended that future organic material of *M. umbilicatum* blooms can be effectively composted at 5% volume in green waste. This compost can be applied as a 1:1 application with soils or potting mixes. Effectively the compost content in plant media is then 2.5% of soil volume, and provides for accelerated growth and reduced stress of native plants and radish crops respectively. This could provide for significant benefits in agriculture as well as for coastal revegetation success. In addition it provides for an efficient and beneficial management option of macroalgal bloom biomass that is otherwise perceived as a public amenity issue.
References


Kikunaga, S., et al. (1999). "The bioavailability of magnesium from Wakame (Undaria pinnatifida) and Hijiki (Hijikia fusiforme) and the effect of alginic acid on magnesium utilization of rats." Plant Foods for Human Nutrition (Formerly Qualitas Plantarum) 53(3): 265-274.


Kikunaga, S., et al. (1999). "The bioavailability of magnesium from Wakame (Undaria pinnatifida) and Hijiki (Hijikia fusiforme) and the effect of alginic acid on magnesium utilization of rats." Plant Foods for Human Nutrition (Formerly Qualitas Plantarum) 53(3): 265-274.


Kikunaga, S., et al. (1999). "The bioavailability of magnesium from Wakame (Undaria pinnatifida) and Hijiki (Hijikia fusiforme) and the effect of alginic acid on magnesium utilization of rats." Plant Foods for Human Nutrition (Formerly Qualitas Plantarum) 53(3): 265-274.


Nabti, E., et al. (2009). "Restoration of growth of durum wheat (Triticum durum var. waha) under saline conditions due to inoculation with the rhizosphere bacterium Azospirillum brasilensis NH and extracts of the marine alga Ulva lactuca." Plant Growth Reg.


## Appendix 1
Compost ‘Totals’ Analysis Report

<table>
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<tr>
<th>Nutrient</th>
<th>Block 1</th>
<th>Block 2</th>
<th>Block 3</th>
<th>Block 4</th>
<th>Block 5</th>
<th>Block 6</th>
<th>Average</th>
</tr>
</thead>
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<tr>
<td>Nitrogen</td>
<td>0.4</td>
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<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Potassium</td>
<td>2.0</td>
<td>2.1</td>
<td>2.2</td>
<td>2.3</td>
<td>2.4</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
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</tr>
<tr>
<td>Copper</td>
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<td>0.2</td>
</tr>
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<td>0.1</td>
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<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
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<tr>
<td>Manganese</td>
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<td>0.4</td>
<td>0.5</td>
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<td>0.3</td>
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<tr>
<td>Iron</td>
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<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Boron</td>
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<td>0.2</td>
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<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Molybdenum</td>
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<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
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<td>0.6</td>
<td>0.2</td>
</tr>
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</tr>
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<td>0.4</td>
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<tr>
<td>Nitrogen : Phosphorus Ratio</td>
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<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Nitrogen : Potassium Ratio</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Carbon : Nitrogen Ratio</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.3</td>
<td>7.4</td>
<td>7.5</td>
<td>7.6</td>
<td>7.7</td>
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</tr>
<tr>
<td>Electrical Conductivity</td>
<td>42.9</td>
<td>43.3</td>
<td>43.7</td>
<td>44.1</td>
<td>44.5</td>
<td>44.9</td>
<td>44.4</td>
</tr>
<tr>
<td>Macropores (%)</td>
<td>69.7</td>
<td>69.9</td>
<td>69.2</td>
<td>69.4</td>
<td>69.7</td>
<td>69.9</td>
<td>69.6</td>
</tr>
<tr>
<td>ECEI</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Submicrons</td>
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<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
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</table>

**Notes:**
1. All analysis is on a dry weight basis, except for pH which is on a wet weight basis.
2. All samples are analyzed on a dry weight basis and are acid washed and leached with 10% HCl.
3. Carbon is measured using a LECO CNS2000 analyzer.
4. Nitrogen: Phosphorus: Potassium ratios are determined on a dry weight basis.
5. All treatments are replicated three times.
6. Samples are analyzed at 10% HCl.
7. All analyses are performed at 10% HCl.
8. All analyses are performed at 10% HCl.