# *Ascophyllum* extract application can promote plant growth and root yield in carrot associated with increased root-zone soil microbial activity

Mohammed Zahidul Alam[1], Gordon Braun[2], Jeffrey Norrie[1], and D. Mark Hodges[2]

*[1]Acadian Seaplants Ltd., 30 Brown Avenue, Dartmouth, Nova Scotia, Canada B3B 1X8 (e-mail: zalam@uoguelph.ca); and [2]Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, 32 Main Street, Kentville, Nova Scotia, Canada B4N 1J5.*
*Received 1 May 2013, accepted 9 October 2013. Published on the web 10 October 2013.*



Alam, M. Z., Braun, G., Norrie, J. and Hodges, D. M. 2014. ***Ascophyllum* extract application can promote plant growth and root yield in carrot associated with increased root-zone soil microbial activity**. Can. J. Plant Sci. **94**: 337–348. Root growth and soil microbial activity were examined in two cultivars of carrot following treatment with *Ascophyllum nodosum* marine-plant extract. Field experiments were established in grower-managed fields of Maverick and Pronto carrots during 2010 and 2011. Soluble *Ascophyllum* extract powder (SAEP) was applied weekly, bi-weekly or tri-weekly at rates of 0 (control), 0.25, 0.50, 0.75 or 1.0 g $L^{-1}$ over 11 to 13 wk. Results indicate that SAEP treatment increased root yields of Maverick and Pronto by about 20 and 15%, respectively, reduced proportion of smaller roots and improved harvest index (HI). Maximum yield was found at or above 0.50 g $L^{-1}$ SAEP for Maverick and at 0.75 g $L^{-1}$ for Pronto. Soil microbial colony counts, respiration and metabolic activity increased following SAEP applications, but varied with SAEP rate and application frequency. Using the Biolog microbial analysis system, maximum average well colour development (AWCD), substrate diversity (H), substrate evenness (E), and substrate richness (S) responses to extract treatment generally showed successive increases at 0.50, 0.75 and 1 g $L^{-1}$ SAEP at tri-weekly application frequencies. With more frequent applications, rates below 1 g $L^{-1}$ led to greater microbial growth, respiration and functional activities. Principal component analysis (PCA) showed a strong relationship between carrot growth, soil microbial populations and activity parameters. These results suggest that seaweed extract application can result in an increase in soil microbial activity associated with increased yield in carrots.

**Key words:** *Ascophyllum* extract, seaweed, carrot, soil microbes, Biolog profile, soil respiration

Alam, M. Z., Braun, G., Norrie, J. et Hodges, D. M. 2014. **L'application d'un extrait d'*Ascophyllum* peut accélérer la croissance et le rendement de la carotte en raison d'une plus grande activité microbienne dans la zone racinaire du sol**. Can. J. Plant Sci. **94**: 337–348. Les chercheurs ont examiné la croissance des racines et l'activité microbienne dans le sol chez deux cultivars de carotte traités avec l'extrait de l'algue marine *Ascophyllum nodosum*. Des parcelles ont été aménagées dans des champs des carottes Maverick et Pronto en 2010 et 2011. De l'extrait en poudre hydrosoluble d'*Ascophyllum* (EPHA) a été appliqué aux parcelles chaque semaine, une semaine sur deux ou une semaine sur trois à raison de 0 (témoin), 0,25, 0,50, 0,75 ou 1,0 g par litre pendant 11 à 13 semaines. Les résultats indiquent que le traitement à l'EPHA augmente le rendement de racines de Maverick et de Pronto d'environ 20 et 15 %, respectivement. Il diminue aussi la proportion de carottes plus petites et rehausse l'indice de récolte. Le rendement optimal a été enregistré à une application d'EPHA supérieure à 0,50 g par litre pour Maverick et de 0,75 g par litre pour Pronto. La numération sur plaque, le taux de respiration et l'activité métabolique des unicellulaires telluriques augmentent après le traitement à l'EPHA, mais varient avec le taux et la fréquence des applications. Quand on recourt au système d'analyse microbienne Biolog, la réaction de la progression colorimétrique maximale moyenne des puits, de la diversité des substrats, de l'uniformité des substrats et de la richesse des substrats au traitement avec l'extrait d'algue progresse généralement de façon successive avec l'application de 0,50, 0,75 et 1 g de PHEA par litre aux trois semaines. Aux fréquences d'application plus élevées, les taux inférieurs à 1 g par litre entraînent une hausse de la croissance, de la respiration et des activités fonctionnelles de la microflore. L'analyse en composantes principales révèle qu'il existe un lien étroit entre la croissance des carottes, la population microbienne du sol et les paramètres de son activité. On en déduit que l'application d'extrait d'algue marine peut accroître l'activité microbienne dans le sol associée à un rendement supérieur en carottes.

**Mots clés:** Extrait d'*Ascophyllum*, algue marine, carotte, microbes du sol, profil Biolog, respiration du sol

The benefits of seaweeds (also known as marine algae) as sources of organic matter and fertilizer nutrients have been known to agriculture for centuries, especially in coastal areas (Khan et al. 2009; Craigie 2011). Extracts from these seaweeds have been used for decades as

**Abbreviations:** **AWCD**, average well colour development; **CFU**, colony-forming units; **CLPP**, community level physiological profile; **E**, substrate evenness; **H**, substrate diversity; **HI**, harvest index; **IAA**, indole-acetic acid; **PAF**, Pseudomonas Agar F; **PCA**, principal component analysis; **RBCC**, Rose Bengal Media; **S**, substrate richness; **SAEP**, soluble *Ascophyllum* extract powder; **SDW**, sterilized deionized water; **TSA**, tryptic soy agar

foliar- and soil-applied treatments in crop production systems due to the presence of a number of plant growth-stimulating compounds (Wally et al. 2012). Unlike chemical fertilizers, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds (Dhargalkar and Pereira 2005). This is one reason why the use of seaweed extracts has gained in popularity in recent years in organic and sustainable agriculture (Craigie 2011). Some unique potential values of *Ascophyllum* extract different from other marine algae and/or other extraction methods have been demonstrated by other researchers (Craigie 2011; Alam et al. 2013). However, the biostimulatory potential of many of these products has not been fully explored in some crops due to a lack of scientific data on active ingredients, synergistic/antagonistic effects and their mode-of-action on plant growth (Khan et al. 2009).

Commercial and academic reports on agricultural uses of seaweed extracts indicate enhanced germination and seedling establishment, increased root growth, nutrient uptake and fruit set, improved resistance to pests and disease, improved resistance to abiotic stresses (e.g., drought, salinity, temperature extremes), and improvements to yield, quality and shelf-life (Blunden 1991; Khan et al. 2009; Craigie 2011). However, results can be inconsistent and can vary with differences in extraction process and seaweed species.

One important root crop that may benefit from seaweed extract treatment is carrot, as one of the most consistent effects of seaweed extract application is the development of a vigorous root system (Metting et al. 1990). Jeannin et al. (1991) suggested that application of seaweed extract is generally most effective on root growth when applied during early vegetative growth, and is consistent with the response to exogenous application of plant growth regulators. Carrots are popular due to their pleasant flavour and overall health benefits related to vitamins, minerals, and fibre (Alasalvar et al. 2005; US Agricultural Statistics 1971). They are a major source of provitamin A, providing 17% of the total vitamin A consumption (Block 1994; Arscott and Tanumihardjo 2010). Production of carrots is also unique as they can grow well on mineral soils containing low organic matter under low temperature and rain-fed conditions (Alasalvar et al. 2005). They have been ranked 10th in terms of nutritional value among 38 other fruits and vegetables, and 7th for their contribution to nutrition (US Agricultural Statistics 1971).

It is well known that crop production can be strongly influenced by soil microbial activity (Killham 1994; Singh et al. 2011). In addition to effects on plant growth and production, the application of seaweed extract may increase microbial growth and activity in the soil, which in turn may lead to improved plant performance (Santoyo et al. 2012). At present, little information is available regarding the influence of seaweed extract application on soil microbes associated with different cropping systems. Studies on the growth and functional activity of complex microbial communities in soil environments remain challenging because of the vast diversity and enormity of the microbial populations (Sun et al. 2004).

The recent development of culture-independent molecular techniques has allowed for a better understanding of the soil microbial community (Garland and Mills 1991; Smit et al. 2001). Using the Biolog community level physiological profile (CLPP) method, it is possible to obtain a general assessment of functional differences in microbial communities from a variety of soil and water samples based on sole-source carbon utilization patterns (Garland and Mills 1991; Zak et al. 1994). Although the exact numbers and taxonomic identities of the bacterial species responsible for these reactions remain unknown, patterns of functional diversity within and among communities can provide meaningful insight into soil community dynamics. Alam et al. (2013) recently reported positive effects of *Ascophyllum nodosum* seaweed extracts on strawberries, as well as benefits to the soil microbial community. Whereas strawberries are valued for their fruit, more information is required regarding the effects of seaweed extracts on the productivity of important root crops.

It is hypothesized in this study that the inclusion of seaweed extracts may lead to improved ''best management practices'' for agroecosystems by benefiting plant–microbe interactions leading to improvements in crop yields and/or quality. This study, therefore, examines the effects of soluble *Ascophyllum* extract powder (SAEP) application on carrot shoot and root growth and effects on the soil microbial community.

## METHODOLOGY

### Study Location, Carrot Varieties and Treatment Application

This 2-yr field study was conducted on two commercial carrot cultivars, Maverick and Pronto (Seed source: NORSECO, 2914 Boulevard Cure-Labelle, Laval, Québec, Canada). A commercially available (Acadian™), water-soluble, alkaline extract powder derived from the brown seaweed *Ascophyllum nodosum* (L.) Le Jolis. was provided by Acadian Seaplants Ltd., Dartmouth, Nova Scotia, Canada (SAEP Batch #5221). The extract contained approximately 1% nitrogen, 0.5% phosphorus, 15% potassium, 0.4% calcium, 0.4% magnesium, 155 ppm iron, 121 ppm manganese, 5 ppm copper, 91 ppm zinc, and 124 ppm boron.

Field studies were conducted during 2010 and 2011 on the premises of Sawler Gardens Ltd., a commercial operation located in Berwick, NS, Canada (lat. 45°2'N, long. 64°44'W, elevation 43 m above sea level). The area is characterized by a silt loam soil and a cool humid temperate climate with a mean annual temperature of 6.9°C and precipitation of about 1116 mm per year.

Small plots (125 $m^2$ excluding the border area) were selected and marked within larger commercial carrot fields (7.0 ha). Overall seeding and management practices were conducted by the grower. During land preparation, wood ash at 7.5 t $ha^{-1}$ and 16–9–30 3B fertilizer at 450 kg $ha^{-1}$ (400 lb $ac^{-1}$) was applied. Maverick and Pronto were seeded during the first and last weeks of May, respectively, in raised single-row-beds at a rate of around 50 seeds per meter of row ( $\sim$2.1 kg $ha^{-1}$). Distance between rows was 61 cm. Seeds took about 3 wk to germinate. *Ascophyllum* extract (SAEP) treatments began 1 wk after emergence and continued until 1 wk before harvest. Each 1-m row of carrot plants (around 45 plants) received 200 mL of 0.25, 0.50, 0.75 or 1.0 g $L^{-1}$ SAEP once a week, once every 2 wk or once every 3 wk. SAEP solutions were prepared by dissolving powdered extract in water and were applied with a hand sprayer on the soil around the shoot. Control treatments received 200 mL of tap water. Border plants separated treated plants by 0.5 m within in the same row and with one untreated row on each side of treated rows to avoid cross contamination. The treatments were arranged in a randomized complete block design with three replications. Standard pest management procedures were followed for both experimental and non-experimental crops using Gesagard 480SC (4 L $ha^{-1}$, active ingredient, Prometryn – 44.5%), Lorex L (3.0 L $ha^{-1}$, active ingredient, Linuron – 480 g $L^{-1}$) and Venture L (1.75 L $ha^{-1}$, active ingredient, Fluazifop-P-butyl and S-isomer – 125 g $L^{-1}$) as herbicides and Bravo 500 (2.5 L $ha^{-1}$, active ingredient, Chlorothalonil – 500 g $L^{-1}$) as fungicide. Crops were harvested 12 to 14 wk after seeding.

After harvest, roots were separated from shoots and washed under tap water. Root length and diameter were recorded for individual roots and graded as $<$10, 10–15, 15.1–20, 20.1–25 and $>$25 cm length and $<$1.0, 1.0–1.5, 1.6–2.0, 2.1–2.5 and $>$2.5 cm diameter. Total root number and fresh weight, and shoot fresh weights were recorded. Malformed roots were counted and weighed. Harvest index (HI) was calculated as the root weight divided by the sum of the root and shoot weight expressed as a percentage.

## Soil Sample Collection

One week before harvest, composite soil samples (eight cores from each of three-replicate 1 m of row per treatment at 0–15 cm in depth and mixed together) were collected only for Maverick during 2010 and 2011. The soils were collected in plastic bags and immediately placed in a Styrofoam cooler containing icepacks for transport to the laboratory. Soil samples were passed through a 2-mm sieve to remove debris. Bacterial colony counts, microbial respiration and Biolog assays were immediately performed on fresh soils.

## Soil Microbial Colony Counts in Response to SAEP Application

Bacterial colony counts were determined by transferring 2 g soil from the composite soil samples into bottles containing 100 mL sterile saline solution (0.85% sodium chloride) and Tween-20 detergent (0.01% wt/vol). The bottles were shaken on an orbital shaker at 150 revolutions per minute for 15–20 min at room temperature. Before sampling, bottles were shaken briefly to re-suspend the soil then allowing $\sim$30 s for heavier particles to settle. One millilitre of the suspension was transferred to a fresh bottle containing 99 mL of sterile saline water and shaken for $\sim$30 s. A 49.2 μL aliquot of the homogenous suspension was spread on petri dishes of tryptic soy agar (TSA), Rose Bengal Media (RBCC, 0.005% chlorotetracycline and 0.005% chloramphenicol) and *Pseudomonas* Agar F (PAF) artificial medium using a spiral plater (Spiral Plater™, Model-D, HZ-60. Spiral Systems Inc. Cincinnati, OH 45244, USA). Plates were incubated at 27 $\pm$ 1°C in the dark and bacterial and fungal colonies were counted under a microscope at 24 h intervals until no new colonies appeared. Fluorescent colonies on PAF were counted under a UV light. The number of bacterial colony-forming units (CFU) per gram of soil was calculated using the dilution factor:

$$Y = b \div (0.0492 \times 100 \times 50)$$

where $Y$ is the number of bacterial CFU per gram of soil and $b$ is the number of bacterial colonies found in each petri dish. The minimum detection limit for this method is $1 \times 10^5$ CFU $g^{-1}$ soil.

## Biolog Community Level Physiological Profile (CLPP) of SAEP-treated Carrot

The bacterial community level physiological profile (CLPP) was determined using Biolog ECO® 96-microwell plates with 31 carbon sources and a control, with three replications per plate. The 2 g soil suspension prepared for colony counts described above was allowed to settle for $\sim$30 s after shaking and 1 mL was then transferred to 99 mL GN-IF (0.4% NaCl, 0.03% Pluronic F-68®, 0.02% Gellum Gum®). After briefly mixing the soil dilution, 150 μL was transferred to each well of the ECO plate and incubated at 27 $\pm$ 1°C in the dark. Colour development was measured at 590 and 750 nm after 24, 48, 72, and 96 h of incubation with an automated Biolog plate reader and data were collected using Microlog 4.01 software (Biolog Inc. 21124 Cabot Blvd. Hayward, CA 94545).

Average well colour development (AWCD) indicating microbial metabolic activity on different substrates was calculated by the method of Garland (1996). Briefly, the absorbance value at 590 nm of the control well was subtracted from all other wells on the plate. To correct for weak false-positive readings, 0.250 absorbance units were subtracted from all well values. Wells with negative values were assigned a value of zero. To compensate for differences in inoculum concentration (normalization),

absorbance values were divided by the average absorbance of all wells that developed colour. Average absorbance was then calculated as the total absorbance of all wells divided by 31 and recorded as the corrected and normalized AWCD for the sample.

Substrate diversity (H), substrate evenness (E) and substrate richness (S) were calculated using the following formulae:

$$H = -\Sigma Pi(\ln Pi)$$

where $Pi$ is the ratio of the activity on a particular substrate to the sum of activities on all substrates.

$$E = H/H\max = H/\log S$$

where $H$ is the substrate diversity and $S$ is the substrate richness.

$$S = H/\log n$$

where $H$ is the substrate diversity and $n$ is the total number of wells that developed colour (Zak et al. 1994).

### Respiration of SAEP-treated soil

For soil respiration studies, 50 g of sieved (2 mm) soil from each treatment and replication including control [blank, 5 mL of sterilized deionized water (SDW)+no soil] was placed into 4 L glass jars in weighing boats. Ten millitres SDW water was used to wet a piece of chromatographic paper inside the jar to prevent the soil from drying. Jar lids were immediately sealed and a blank sample was measured as time zero ($T=0$) value for $CO_2$ concentration. Carbon dioxide concentrations evolved from the same soil samples were measured every 24 h for 5 d and expressed as the percent $CO_2$ using an ICA 41, 42, 43 modular gas analyzer (International Controlled Atmosphere Ltd., Kent, UK).

The dry weight of each sample was determined at the end of the experiment by drying in an oven at 60°C for 5 d. The dried soil weight was used to calculate the rate of $CO_2$ production expressed as mg $CO_2$ $kg^{-1}$ dry soil $d^{-1}$.

### Statistical Analysis

Field experimental data were analyzed as a two-factor (SAEP application rate and timing) randomized complete block design with three replications. Data were subjected to analysis of variance using GenStat 11.1 (VSN International, Hemel Hampstead, UK) and expressed as linear or curvilinear regressions ($P<0.05$). When $F$ was significant at the $P<0.05$ level, treatment means were separated using the least significant difference test (LSD). Contrast analyses were used to test the similarity of regression responses. In the absence of a significant site–year interaction, carrot yield and growth data were analyzed as the average of 2 yr (2010 and 2011). In the absence of significant SAEP application timings or SAEP rate × application timing interactions, data were averaged over SAEP application timings. Microbial colony counts data were analyzed separately for each of the 2 yr as the site × year interaction was significant. For Biolog studies, in the absence of significant SAEP application timings or SAEP rate × application timing interactions; results for application frequencies were averaged. Principal component analysis (PCA) was conducted to evaluate the relationship between the treatments (SAEP rate × application timing) with the number of roots, root weight, shoot weight, total biomass, percent deformed roots, percent roots <15 cm in length, percent roots >15 cm, percent roots <2 cm in diameter, percent roots >2 cm, $\log_{10}$ bacterial CFU $g^{-1}$ soil on PAF (*Pseudomonads*), $\log_{10}$ bacterial CFU $g^{-1}$ soil on TSA, $\log_{10}$ fungal CFU $g^{-1}$ soil on RBCC and AWCD, diversity, evenness, and richness from Biolog data using GenStat 11.1.

## RESULTS

### Shoot Fresh Weight, Root Fresh Weight, Total Biomass Yield and Harvest Index

Combined data from 2010 and 2011 showed that *Ascophylum* extract rates had significant effects on root and total biomass yields (Fig. 1a and b), harvest indices (Fig. 2), <10 and 10–15 cm length roots (Fig. 3a) and <1.0 and 1.0–1.5 cm diameter roots (Fig. 3b) for both Maverick and Pronto. SAEP application frequency or interaction between SAEP rates and application frequency were not statistically significant ($P>0.05$); therefore data were averaged over all application frequencies.

Significant quadratic increases in root ($P=0.0015$) and total biomass yields ($P=0.0014$) of Maverick were observed when plants were treated with 0.25 and 0.50 g $L^{-1}$ SAEP compared with the control (Fig. 1a). However, 0.75 and 1.0 g $L^{-1}$ SAEP rates resulted in yields comparable with the 0.50 g $L^{-1}$ rate. For Pronto, root ($P=0.0011$) and total biomass yields ($P=0.0018$) increased linearly in response to SAEP treatments up to the 0.75 g $L^{-1}$ rate (Fig. 1b). Effects of 1.0 g $L^{-1}$ SAEP were not significantly ($P=0.15$) different from 0.25 g $L^{-1}$ and the control.

Quadratic increases in harvest indices were observed for both Maverick ($P=0.003$) and Pronto ($P=0.007$); however, the rate of increase in HI was notably slower for Pronto and there was no sign of a plateau up to 1 g $L^{-1}$ (Fig. 2). For Maverick, HI gradually increased up to 0.75 g $L^{-1}$ compared with the control. The 1.0 g $L^{-1}$ rate was not significantly different ($P=0.083$) from the 0.25 and 0.50 g $L^{-1}$ SAEP rates.

### Root Length, Diameter, Malformation

Effects of SAEP treatments were significant only on shorter length roots (>10 and 10–15 cm; $P<0.001$ for both). Results showed that the percentage of roots <10 and 10–15 cm long gradually decreased compared with the control with increasing rates of SAEP treatments up to 0.5 g $L^{-1}$ (Fig. 3a). The trends of decreases were

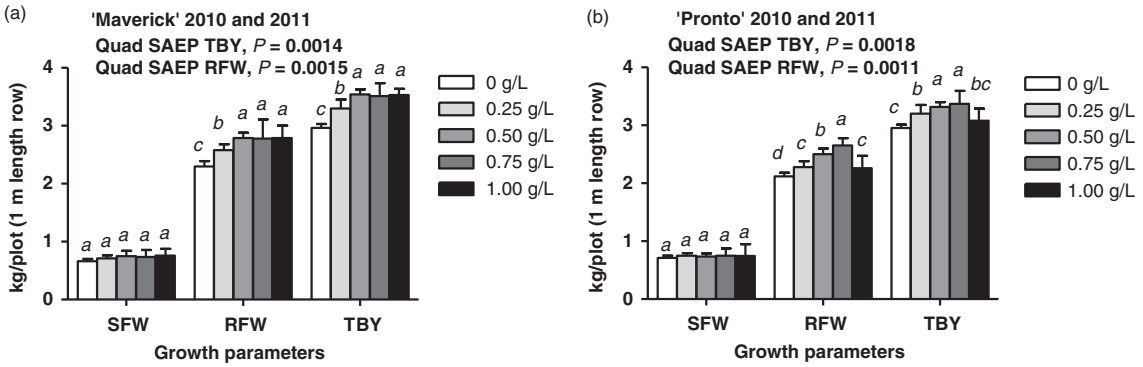


**Fig. 1.** Effect of soluble *Ascophyllum* extract powder (SAEP) on shoot fresh weight (SFW), root fresh weight (RFW) and total biomass yield (TBY) of Maverick (a) and Pronto (b). The bars represent mean values (±SE) of three replicates over the 2 yr. Each dependent variable was analyzed separately. Means with different lower case letters are significantly different ($P < 0.05$) between SAEP rates.



more or less similar for both varieties. Further increases in SAEP rates gave results comparable with the 0.5 g $L^{-1}$ rate. Similar to root length, the effects of SAEP treatments were significant for smaller root diameters (<1.0 and 1.0–1.5 cm). Results show that the percentage of roots with diameters <1.0 and between 1.0–1.5 cm gradually decreased with increasing rates of SAEP treatments up to 0.5 g $L^{-1}$ (Fig. 3b). Decreasing trends were more or less similar for both varieties. Further increases in SAEP rates caused an increase in smaller diameter roots comparable with the 0.5 g $L^{-1}$ rate. There was no significant difference ($P = 0.071$ and 0.246, respectively, for Maverick and Pronto) in percent malformed roots in response to soil treatments with SAEP in either cultivar or year (data not shown).

## Microbial Colony Counts

Only Maverick was selected for microbial growth and activity investigation in response to SAEP treatments during 2010 and 2011. Microbial colony counts on media (PAF, TSA, and RBCC) varied greatly in response to different SAEP rates and timings (Fig. 4a and b). On average, SAEP treatments of 0.25, 0.50, 0.75 and 1.0 g $L^{-1}$ increased bacterial populations isolated on PAF by 7, 29, 38 and 39%, respectively, compared with the non-treated control. Total culturable bacterial populations (isolated on TSA) increased 30, 37, 39 and 35%, respectively, in response to applications of 0.25, 0.50, 0.75 and 1.0 g $L^{-1}$ of SAEP. Fungal populations

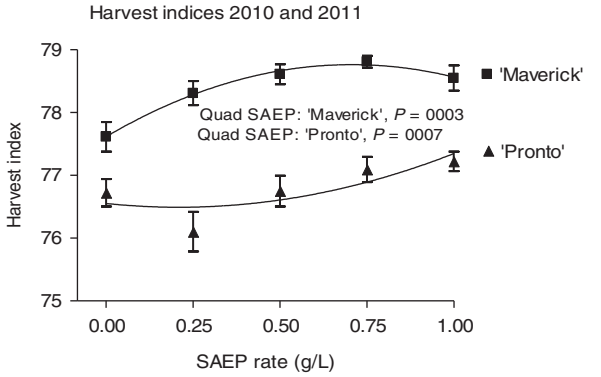


**Fig. 2.** Effect of soluble *Ascophyllum* extract powder (SAEP) on harvest indices of Maverick and Pronto. Data points indicate mean replicate ($n = 6$) harvest index values (±SE) over the 2 yr (2010 and 2011). $R^2$ values for both regression lines are ≤0.95.

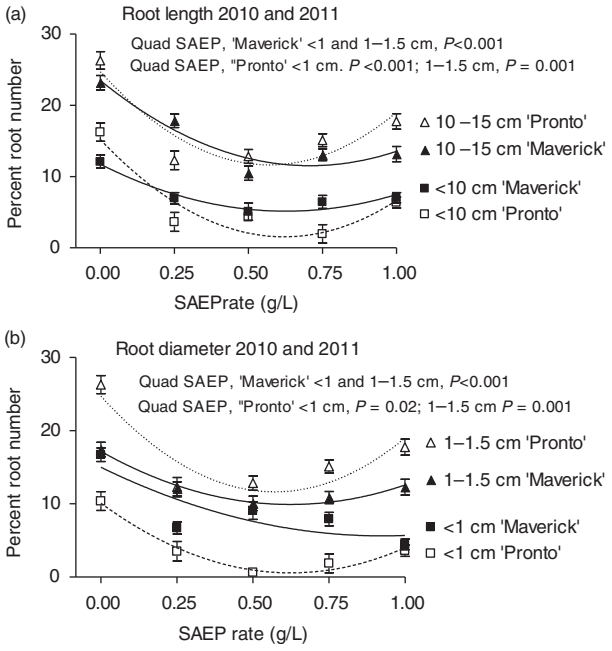


**Fig. 3.** Effect of soluble *Ascophyllum* extract powder (SAEP) on root length (a) and diameter (b) of Maverick and Pronto during 2010 and 2011. Data are presented as mean (replicate and year) % root numbers <10 and 10–15 cm length and <1 and 1.5 cm diameter (±SE). $R^2$ values for all regression lines are ≤0.95.

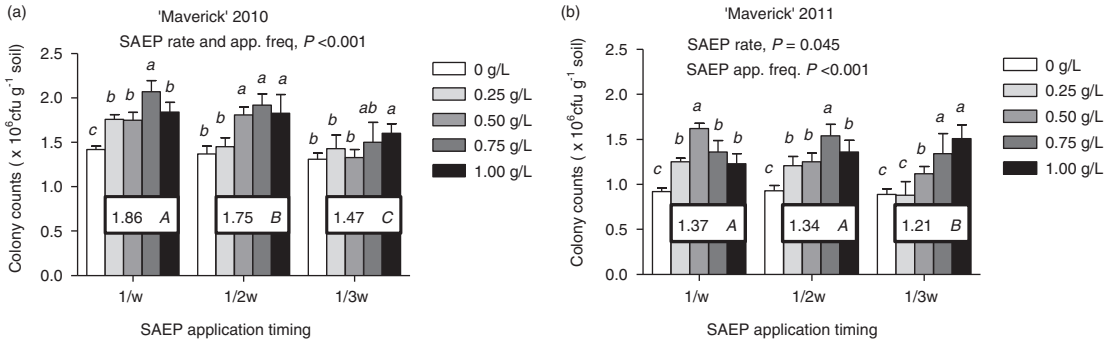


**Fig. 4.** Effect of soluble *Ascophyllum* extract powder (SAEP) on total microbial (bacteria and fungi) colony counts of Maverick during 2010 (a) and 2011 (b). The bars represent mean ($n = 3$) total colony counts $\pm$ SE. Data were analyzed separately for each of the 2 yr. Boxed values on the bars indicate total colony counts across all SAEP rates (excluding control). Means with different lower or upper case letters are significantly different at $P < 0.05$.

isolated on RBCC medium showed increases of 6, 11, 16 and 9% in soils treated with 0.25, 0.50, 0.75 and 1.0 g $L^{-1}$ of SAEP, respectively (data not presented).

During the 2010 season, at an application frequency of once per week, all application rates of SAEP exhibited higher microbial colony counts compared with the water-treated control with the highest counts at a rate of 0.75 g $L^{-1}$ (Fig. 4a). Responses at 0.25, 0.50 and 1.0 g $L^{-1}$ were lower and not significantly ($P > 0.05$) different from each other. At an application frequency of once every 2 wk microbial colony counts were statistically equivalent to 0.50, 0.75 and 1.0 g $L^{-1}$, but greater than colony counts for 0.25 g $L^{-1}$ or the control. Application frequencies of once every 3 wk recorded a significant ($P < 0.001$) increase in microbial population numbers for only the 1.0 g $L^{-1}$ rate of SAEP. On average, colony counts in 2010 were greater when soil was treated once per week ($1.86 \times 10^6$ CFU $g^{-1}$ soil) followed by bi-weekly applications ($1.75 \times 10^6$ CFU $g^{-1}$ soil) and tri-weekly applications ($1.47 \times 10^6$ CFU $g^{-1}$ soil) (Fig. 4a).

During 2011, soil microbial colony counts were 25% lower than in 2010 (Fig. 4b). At an application frequency of once per week, the 0.50 g $L^{-1}$ SAEP treatment had the highest colony counts. Rates of 0.25, 0.75 and 1.0 g $L^{-1}$ were comparable and greater than the control. Bi-weekly applications of 0.75 g $L^{-1}$ SAEP had the highest colony counts while 0.25, 0.50 and 1.0 were equivalent but significantly ($P < 0.05$) greater than the control. Tri-weekly application of SAEP resulted in the highest colony counts at 0.75 and 1.0 g $L^{-1}$. The 0.25 g $L^{-1}$ rate was comparable to the control and lower than the 0.5 g $L^{-1}$ rate. Unlike 2010, there was no significant difference in average microbial colony counts when soil was treated weekly or bi-weekly ($1.37 \times 10^6$ and $1.34 \times 10^6$ CFU $g^{-1}$ of soil, respectively). However, responses were greater for weekly and bi-weekly applications compared to tri-weekly applications ($1.21 \times 10^6$ CFU $g^{-1}$ of soil) (Fig. 4b).

## Biolog Analysis

The metabolic activities of the soil microbes measured on different carbon substrates were characterized using average well colour development (AWCD), substrate diversity (H), substrate evenness (E) and substrate richness (S) measured at 24, 48 72, and 96 h incubation periods. Only data collected at the 48 h time interval was used because after this interval bacteria growth has overwhelmed the capacity of the plate and reached the maximum absorbance in most wells. All the data presented are statistically significantly different at $P < 0.05$. In the absence of significant differences, results for application frequencies of once per week, once per 2 wk and once per 3 wk were averaged.

The AWCD, H, E and S of all carbon sources generally showed linear responses to different SAEP rates during 2010 and interactions between SAEP rates and application frequency were significant only for AWCD (Fig. 5). Weekly application of SAEP led to increases in AWCD compared with water-treated soil with the highest AWCD at 1.0 g $L^{-1}$. There were no significant differences between 0.25, 0.50 and 0.75 g $L^{-1}$ (Fig. 5a). Bi-weekly applications of 0.25 g $L^{-1}$ SAEP were comparable with water-treated soil and 0.50, 0.75 and 1.0 g $L^{-1}$ had significantly higher and similar AWCD responses. With tri-weekly applications, the 1.0 g $L^{-1}$ rate had the highest AWCD response and there was no significant difference between 0.50 and 0.75 g $L^{-1}$. On average, AWCD was higher when soil was treated weekly or bi-weekly compared with tri-weekly (Fig. 5e). For H, E and S variables, all SAEP treatments led to increases with 1.0 g $L^{-1}$ ASEP being the highest and generally 0.25, 0.50 and 0.75 g $L^{-1}$ showing similar effects (Fig. 5b, c and d).

During 2011, the AWCD, H, E and S of all carbon sources also showed general linear responses to different SAEP rates (Fig. 5e, f, g and h) and interactions between SAEP rates and application frequency were significant for AWCD, H and E (Fig. 5e, f and g). In general, weekly applications of 0.50 and 0.75 g $L^{-1}$ SAEP had

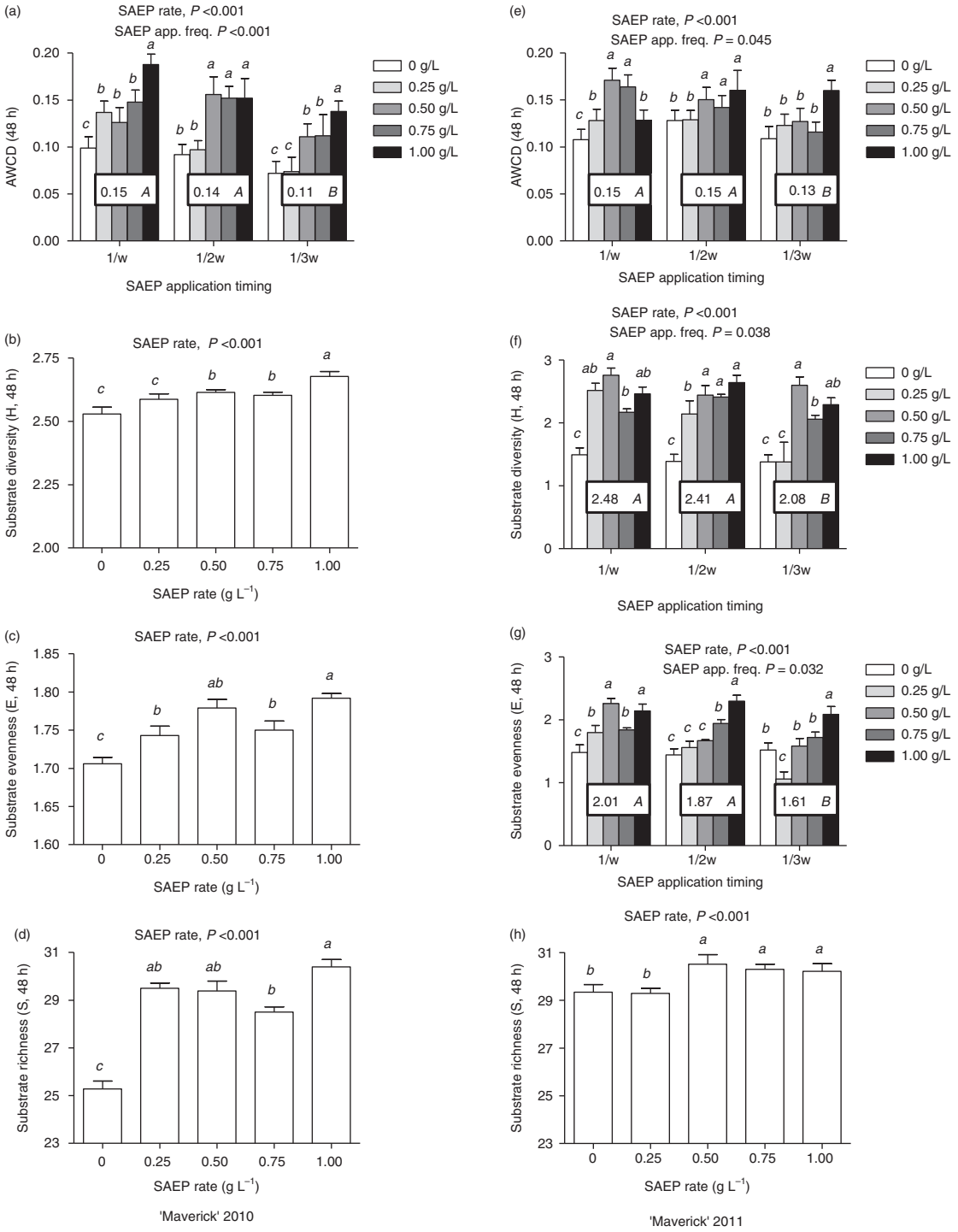


**Fig. 5.** Effect of soluble *Ascophyllum* extract powder (SAEP) on average well colour development (AWCD) (a and e), substrate diversity (b and f), substrate evenness (c and g) and substrate richness (d and h) of Maverick during 2010 and 2011. The bars represent mean replicate ($n = 3$) values $\pm$ SE. Boxed values on the bars indicate mean values across all SAEP rates (excluding control). Means with different lower or upper case letters are significantly different at $P < 0.05$.

greater responses for AWCD, H and E. At 1.0 g $L^{-1}$, the responses reduced or remained similar to 0.50 and 0.75 g $L^{-1}$ SAEP (Fig. 5e, f and g). Bi- or tri-weekly rates of 0.25 g $L^{-1}$ had similar H and E responses as control, 0.50, 0.75 and 1.0 g $L^{-1}$ rates generally increased the responses usually being highest at 1.0 g $L^{-1}$

(Fig. 5e, f and g). AWCD, H and E responses were similar and higher when soil was treated once every week or once per 2 wk compared with tri-weekly applications (Fig. 5e, f and g). For S responses, 0.25 g $L^{-1}$ showed no response. However, 0.50, 0.75 and 1.0 g $L^{-1}$ rates had similar and greater responses compared to the control (Fig. 5h).

### Soil Respiration

Combined data from 2010 and 2011 showed that weekly applications of 0.50 g $L^{-1}$ SAEP had the highest soil respiration measurements while 0.75 and 1.0 g $L^{-1}$ SAEP had the lowest respiration measurements (Fig. 6a). Bi-weekly application of 0.50 g $L^{-1}$ SAEP also had the highest respiration measurement while all other treatments were similar (Fig. 6b). With tri-weekly applications, all SAEP rates exhibited higher respiration measurements compared with the water-treated soil with the highest respiration at 0.50 g $L^{-1}$ (Fig. 6c).

### Principal Component Analysis

The PCA explains 59% of the variation between the treatments with 42% on Score 1 and 17% on Score 2. The rate and frequency of SAEP application data points are spread along Score 1 from a negative score to a positive score with the lowest application rate on the negative and the highest on the positive (Fig. 7). The frequency of application does not appear to have an influence on this distribution but the rate of SAEP applied does. The shoot weight, root weight, total biomass, reduction in roots with diameters $<2$ cm, reduction in roots with a length $<15$ cm, bacterial population numbers on PAF (selective for *Pseudomonads*) and TSA (general bacteria) and bacterial AWCD are all strongly affected by higher rates of SAEP application with a positive score where the lower rates have a larger $>2$ cm diameter and $>15$ cm root length. The total number of roots, percent of deformed roots, fungal population numbers, bacterial diversity, evenness and richness are not strongly affected by the SAEP application rate or frequency of SAEP application as reflected in the low score 2 (17%) with little treatment response spread along score 2.

## DISCUSSION

This study investigated the effects of *Ascophyllum* extract powder (SAEP) treatment on carrot yield and root-zone microbial communities of two carrot cultivars, Maverick and Pronto. SAEP applications increased root yields (fresh weight) of Maverick and Pronto by about 20 and 15%, respectively. The greatest root yields were recorded when plants were treated with 0.50 or 0.75 g $L^{-1}$ SAEP for both varieties irrespective of application frequency. Similarly, SAEP applications also increased the total soil microbial colony counts, microbial physiological activity and soil respiration with maximum response at 0.50 or 0.75 g $L^{-1}$ of SAEP. This was particularly true for soil bacterial populations. Seaweed extracts from marine algae contain major and minor nutrients and bioactive substances with beneficial effects that enhance root and shoot growth, yield and quality in many agricultural and horticultural crops (Abdel-Hafeez 2005; Zhang and Ervin 2008; Fan et al. 2011). Brown seaweeds contain sterols (an essential group of lipids) primarily fucosterol

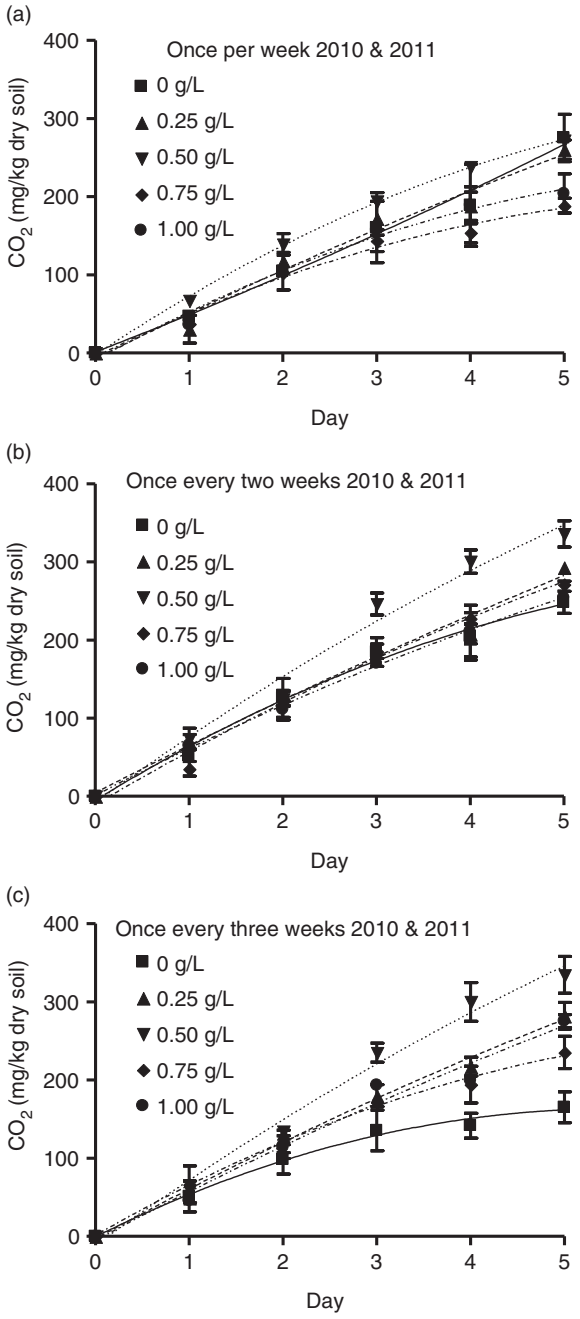


**Fig. 6.** Effect of soluble *Ascophyllum* extract powder (SAEP) on soil respiration of Maverick applied once per week (a), once every 2 wk (b) and once every 3 wk (c). Data are presented as mean ($\pm$SE) respiration over replicates and year (2010 and 2011). $R^2$ values for all regression lines are $\leq 0.95$.

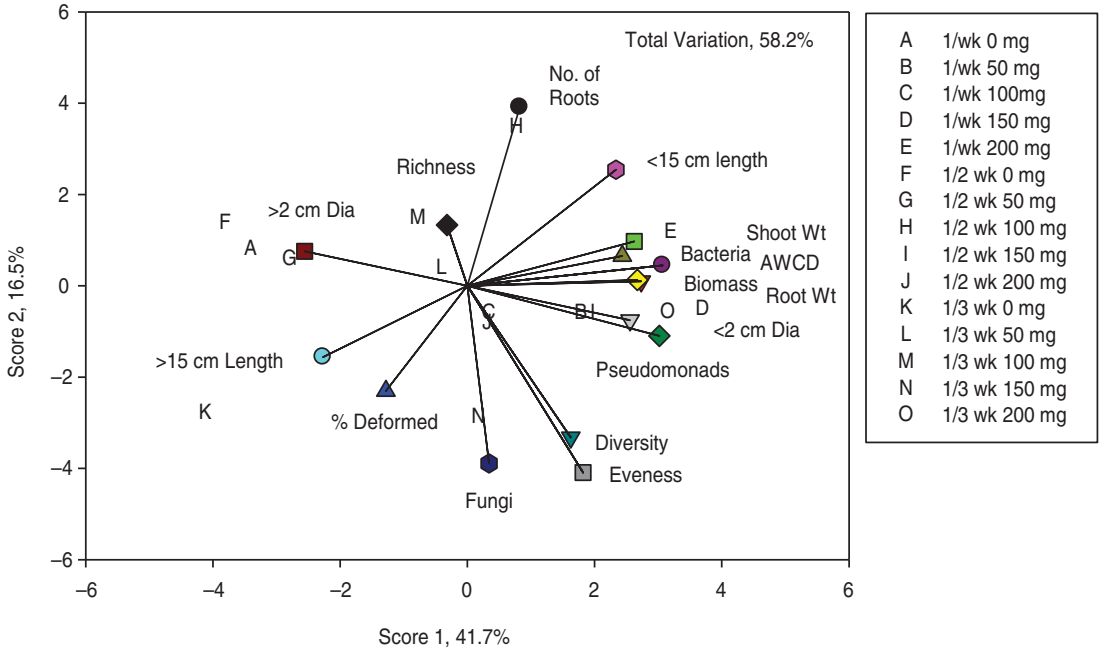


**Fig. 7.** Principal component analysis showing the effects of soluble *Ascophyllum* extract powder (SAEP) application on carrot growth and soil microbial activities. Variables shown include number of roots, fresh root weight, fresh shoot weight, total biomass, percent deformed roots, percent roots <15 cm in length, percent roots >15 cm, percent roots <2 cm in diameter, percent roots >2 cm, log10 bacterial colony-forming units (CFU) per gram soil on Pseudomonas Agar F (*Pseudomonads*), log10 bacterial CFU per gram soil on tryptic soy agar, log10 fungal CFU per gram soil on Rose Bengal Media and average well colour development, substrate diversity, substrate evenness, and substrate richness from Biolog plates as affected by all combinations of five rates of SAEP and three frequencies of application. The first two axes explain 59% of the relationship between the measured variables (carrot growth and microbial populations) and treatment (SAEP rate and application frequency).

and fucosterol derivatives that form essential components of plant cell walls (Govindan et al. 1993). These compounds have been shown to positively affect growth of plant shoot and root tissues (Zhang et al. 2003; Zhang and Ervin 2004) and have been reported to stimulate the growth and yield of plants (Khan et al. 2009). Marine algal extracts are reportedly rich in cytokinins, auxins and auxin-like compounds (Stirk et al. 2003; Craigie 2011) and have been found to stimulate endogenous hormone production (Wally et al. 2012). In general, cytokinins and auxins are strong promoters of root growth, stimulating cell division and differentiation. The *Ascophyllum* extract used in this study contained some essential plant nutrient elements (mentioned in the Methodology section). However, as SAEP was diluted to 0.25, 0.50, 0.75 and 1.0 g $L^{-1}$ prior to application, the nutrient concentrations were low relative to the crop requirements and not expected to significantly impact growth relative to the regular fertility program. Conversely, bioactive compounds such as indole-acetic acid (IAA) found in seaweed extracts can be effective at very low concentrations. Concentrations in the range of $10^{-9}$ to $10^{-12}$ M stimulated root growth while higher concentrations inhibited growth (Patten and Glick 2002). Likewise, a 1:600 dilution of kelp extract with water stimulated tomato root growth while a 1:100 dilution inhibited root growth (Finnie and van Staden 1985). More recently, *A. nodosum* extract were shown to affect the root growth of *Arabidopsis* at very low concentrations (0.1 g $L^{-1}$) whereas plant height and number of leaves increased using 1 g $L^{-1}$ treatments (Rayorath et al. 2008). In our study, SAEP applications were started 1 wk after seedling emergence. Increases in root length and diameter of carrot were greater at 0.5 g $L^{-1}$ and 0.75 g $L^{-1}$ compared with the control and 1.0 g $L^{-1}$ rate (Fig. 3a and b). However, applications of 1.0 g $L^{-1}$ reduced carrot growth and bacterial populations compared with 0.75 g $L^{-1}$ applications. The possible reasons may include the bioactive components of *Ascophyllum* extracts which at higher concentrations can inhibit plant growth. *Ascophyllum* extracts contain biostimulants including common higher plant hormones such as auxins, cytokinins and abscisic acid (Zhang et al. 1993; Tarakhovshaya et al. 2007) and the modern concept of plant hormones suggests that bioactive compounds can influence physiological process at low concentrations and can inhibit at higher concentration (Davies 2004).

Success in yield improvement by modern breeding has been mainly achieved through improvement in HI (Kawano 1990). While biomass yield represents the crop's accumulated photosynthesis, HI represents

the efficiency of the crop to convert photosynthesized products into an economically valuable form. Our results show that SAEP applications increased HI (Fig. 2). To our knowledge, this is the first report of seaweed extract improving photosynthate partitioning or HI.

Considerable evidence has been published demonstrating the growth-promoting activities of common soil bacteria (Lugtenberg et al. 1991; Bloemberg and Lugtenberg 2001; Patten and Glick 2002). Soil bacteria such as *Pseudomonas* and *Bacillus* species produce cytokinin-like substances, gibberellin and IAA. Indoleacetic acid production by bacteria has been demonstrated to cause the same root growth stimulation as exogenously applied IAA. In addition, 83% of endophytic bacteria isolated from carrot roots were effective plant growth promoters (Surette et al. 2003). We investigated the response of soil microbial communities to several SAEP applications. Microbial metabolic activity, substrate diversity, richness, or evenness provides insight into functional aspects of biodiversity among different treatments (Girvan et al. 2003). Average well colour development (AWCD) is an indicator of the extent of microbial metabolic activity. Substrate richness (S) is the number of different substrates utilized by a microbial community. Substrate evenness (E) measures the equitability of activities across all utilized substrates. Substrate diversity (H) consists of species richness, the total number of species present, species evenness and the distribution of species (Trevors 1998; Overas 2000). Our results show that the highest colony counts, respiration and AWCD, H, E, and S values were most often found following 0.50 or 0.75 g $L^{-1}$ SAEP applications weekly or bi-weekly. While this was generally true for the overall microbial populations measured in this study, there were some interesting differences among member groups of the microbial community. *Pseudomonas* Agar F is semi-selective medium for Pseudomonads and more particularly fluorescent Pseudomonads. However, in our study the numbers of fluorescent Pseudomonads on soil dilution plates was low and could not be accurately quantified. The number of colonies of non-fluorescent bacteria on PAF was significantly increased at 0.75 and 1.0 g $L^{-1}$ SAEP. Bacteria in the genus *Pseudomonas* have been well established as making up a large part of the plant growth promoting Rhizobacteria (Surette et al. 2003). The largest increase in bacterial colony numbers was observed on TSA from soils treated with 0.75 g $L^{-1}$ of SAEP. Surette et al. (2003) also observed that the largest numbers of endophytic bacteria in carrot were isolated on TSA. Fungal population numbers on RBCC media only increased slightly at 0.75 g $L^{-1}$ SAEP. Interestingly, fungi are poor colonizers of the root rhizosphere (Lugtenberg et al. 1991) and this may explain their limited response to SAEP applications. Also, Alam et al. (2013) demonstrated that soil microbial respiration was not increased in response to SAEP applications in the absence of plant roots. This may suggest that SAEP applications are stimulating root growth which in turn is stimulating rhizobacterial growth. The possibility that SAEP does not directly stimulate rhizobacterial population growth does not indicate that bacteria are not having a beneficial effect on plant growth. Surette et al. (2003) have shown that common *Pseudomonas* and *Bacillus* species present in Nova Scotia soils form beneficial endophytic relationships with carrots. It has been well established in other ecosystems that plants interact intimately with soil microorganisms that contribute to soil fertility, plant nutrition and to overall plant health (Sun et al. 2004; Singh et al. 2011).

The observation that bacterial populations and carrot growth have similar responses to increasing concentrations of SAEP suggests that there may be a relationship between them. A cause and effect relationship between increased root growth and increased soil bacterial populations and activity in response to SAEP application is difficult to determine but will be the subject of future research. *Pseudomonas* and *Bacillus* species are common soil bacteria that have been identified as endophytes of carrot roots (Surette et al. 2003). This very close relationship between bacteria and carrot roots makes it difficult to determine if the SAEP is stimulating root growth which encourages bacterial population increase or if SAEP stimulates bacterial growth that in turn stimulates root growth.

In summary, this study examined the effects of *Ascophyllum* extract (SAEP) application on carrot root productivity and soil microbial dynamics. Our results show a significant positive effect of SAEP application on root yield and soil microbial numbers and activity. The stimulation of root yield, and microbial activity observed in this study may be due in part to the bioactive components in SAEP. The modern concept of plant hormones suggests that bioactive compounds influence physiological processes at concentrations, far below those required for minerals and micronutrients (Davies 2004). Marine algae, including brown seaweeds, are known to contain biostimulants including common higher plant hormones such as auxins, cytokinins and abscisic acid (Zhang et al. 1993; Tarakhovshaya et al. 2007). Moreover, it has been demonstrated that common soil bacteria produce phytohormones including IAA (Dodd et al. 2010). The response of carrot root growth was consistent with the kind of response expected with IAA applications. The relationship between bacteria and plant roots makes it difficult to determine whether the observed growth response was due to SAEP, the bacteria or both. Considering current views on the interconnectedness and interdependence of all components in an ecosystem, we propose that the observed responses were due to both SAEP and soil microbial communities (Bonfante and Anca 2009; Tikhonovich and Provorov 2011). However, a noteworthy result of this study is that storage root yield of carrot was increased by the addition of small amounts of a natural product.

## ACKNOWLEDGEMENT

The research was supported by the Nova Scotia Technology Development Program, NS, Canada; Agriculture and Agri-Food Canada (AAFC); and Acadian Seaplants Ltd. The authors are very thankful to Sawler Garden Ltd., Berwick, NS for allowing us to conduct this research in their fields and providing in-kind support. The authors also would like to thank Sherry Fillmore, Jeff Franklin, Michele Elliot and Ashley Smith for technical assistance.



**Abdel-Hafeez, A. A. 2005.** Effect of pre-harvest spraying with seaweed extract ''Acadian'' and active dry yeast on ''Le Conte'' pear (*Pyrus leconte*, Rehd) fruit quality and cold storability. Ann. Agric. Sci. Moshtohor. **43**: 1915–1935.

**Alam, M. Z., Braun, G., Norrie, J. and Hodges, D. M. 2013.** Effect of *Ascophyllum* extract on plant growth, fruit yield and soil microbial communities of strawberry. Can. J. Plant Sci. **93**: 23–36.

**Alasalvar, C., Al-Farsi, M., Quantick, P. C., Shahidi, F. and Wiktorowicz, R. 2005.** Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory qualities of ready-to-eat shredded orange and purple carrots. Food Chem. **89**: 69–76.

**Arscott, S. A. and Tanumihardjo, S. A. 2010.** Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. Comp. Rev. Food Sci. Food Safety **9**: 223–239.

**Block, B. 1994.** Nutrient sources of provitamin A carotenoids in the American diet. Am. J. Epidemiol. **139**: 290–293.

**Bloemberg, G. V. and Lugtenberg, B. J. J. 2001.** Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr. Opin. Plant Biology. **4**: 343–350.

**Blunden, G. 1991.** Agricultural uses of seaweeds and seaweed extracts. Pages 65–81 *in* M. D. Guiry and G. Blunden, eds. Seaweed resources in Europe: uses and potential. Wiley, Chichester, UK.

**Bonfante, P. and Anca, I. 2009.** Plants, mycorrhizal fungi, and bacteria: A network of interactions. Ann. Review Microbiol. **63**: 363–383.

**Craigie, J. S. 2011.** Seaweed extract stimuli in plant science and agriculture. J. Appl. Phycol. **23**: 371–393.

**Davies, P. J. 2004.** Plant hormones, biosynthesis, signal transduction, action! Vol. 3, 3rd edn. Kluwer, Dordrecht, the Netherlands. 750 pp.

**Dhargalkar, V. K. and Pereira, N. 2005.** Seaweed: promising plant of the millennium. Sci. Culture **71**: 60–66.

**Dodd, I. C., Zinovkina, N. Y., Safronova, V. I. and Belimov, A. A. 2010.** Rhizobacterial mediation of plant hormone status. Ann. Appl. Biol. **157**: 361–379.

**Fan, D., Hodges, D. M., Zhang, J., Kirby, C. W., Ji, X., Locke, S. J., Critchley, A. T. and Prithiviraj, B. 2011.** Commercial extract of the brown seaweed *Ascophyllum nodosum* enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects *Caenorhabditis elegans* against oxidative and thermal stress. Food Chem. **124**: 195–202.

**Finnie, J. F. and van Staden, J. 1985.** Effects of seaweed concentrate and applied hormones on *in vitro* cultured tomato roots. J. Plant Physiol. **120**: 215–222.

**Garland, J. L. 1996.** Patterns of potential C source utilization by rhizosphere communities. Soil Biol. Biochem. **28**: 223–230.

**Garland, J. L. and Mills, A. L. 1991.** Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. **57**: 2351–2359.

**Girvan, M. S., Bullimore, J., Pretty, J. N., Osborn, A. M. and Ball, A. S. 2003.** Soil type is the primary determinant of the composite of the total and active bacterial communities in arable soils. Appl. Environ. Microbiol. **69**: 1800–1809.

**Govindan, M., Hodge, J. D., Brown, K. A. and Nunez-Smith, M. 1993.** Distribution of cholesterol in Caribbean marine algae. Steroids **58**: 178–180.

**Jeannin, I., Lescure, J. C. and Morot-Gaudry, J. F. 1991.** The effects of aqueous seaweed sprays on the growth of maize. Bot. Mar. **34**: 469–473.

**Kawano, K. 1990.** Harvest index and evoluation of major food crop cultivars in the tropics. Euphytica **46**: 195–202.

**Khan, W., Rayirath, U. P., Jithesh, M. N. S. S., Rayorath, P., Hodges, D. M., Critchley, A. T., Craigie, J. S., Norrie, J. and Prithiviraj, B. 2009.** Seaweed extracts as biostimulants of plant growth and development. J. Plant Growth Regul. **28**: 386–399.

**Killham, K. 1994.** Soil ecology. Cambridge University Press, Cambridge, UK.

**Lugtenberg, B. J. J., de Weger, L. A. and Bennett, J. W. 1991.** Microbial stimulation of plant growth and protection from disease. Curr. Opin. Biotechnol. **2**: 457–464.

**Metting, B., Zimmerman, W. J., Crouch, I. J. and van Staden, J. 1990.** Pages 589–627 *in* I. Akastsuka eds. Agronomic uses of seaweed and microalgae. Introduction to applied physiology. SPB Academic Publishing, The Hague, the Netherelands.

**Overas, L. 2000.** Population and community level approaches for analysing microbial diversity in natural environments. Ecol. Lett. **3**: 236–251.

**Patten, C. L. and Glick, B. R. 2002.** Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. App. Environ. Microbiol. **68**: 3795–3801.

**Rayorath, P., Narayanan, J. M., Farid, A., Khan, W., Palanisamy, R., Hankins, S., Critchley, A. T. and Prithiviraj, B. 2008.** Rapid bioassays to evaluate the plant growth promoting activity of *Ascophyllum nodosum* (L) Heynh. J. Appl. Phycol. **20**: 423–429.

**Santoyo, G., Orozco-Mosqueda, Ma. del, C. and Govindappa, M. 2012.** Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: a review. Biocontrol Sci. Technol. **22**: 855–872.

**Singh, J. S., Pandey, V. P. and Singh, D. P. 2011.** Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. Agric. Ecosys. Environ. **140**: 339–353.

**Smit, E., Leeflang, P., Gommans, S., van den Broek, J., van Mil, S. and Wernars, K. 2001.** Diversity and seasonal fluctuations of the dominant members of the bacterial soil community wheat field as determined by cultivation and molecular methods. Appl. Environ. Microbiol. **67**: 2284–2291.

**Stirk, W. A., Novak, M. S. and van Staden, J. 2003.** Cytokinins in macroalgae. Plant Growth Regul **41**: 13–24.

**Sun, H. Y., Deng, S. P. and Raun, W. R. 2004.** Bacterial community structure and diversity in a century-old manure-treated agroecosystem. App. Environ. Microbiol. **70**: 5868–5874.

**Surette, M. A., Sturz, A. V., Lada, R. R. and Nowak, J. 2003.** Bacterial endophytes in processing carrots (*Daucus carota* L.

var. *sativus*): their localization, population density, biodiversity and their effects on plant growth. Plant Soil **253**: 381–390.
**Tarakhovshaya, E. R., Maslov, Y. I. and Shishhova, M. F. 2007.** Phytohormones in algae. Russ. J. Plant Physiol. **54**: 163–170.
**Tikhonovich, I. A. and Provorov, N. A. 2011.** Microbiology is the basis of sustainable agriculture: an opinion. Ann. Appl. Biol. **159**: 155–168.
**Trevors, J. T. 1998.** Bacterial biodiversity in soil with an emphasis on chemically-contaminated soils. Water Air Soil Pollut. **101**: 45–67.
**US Agricultural Statistics. 1971.** US agricultural statistics. US Department of Agriculture, Washington, DC.
**Wally, O. S. D., Critchley, A. T., Hiltz, D., Craigie, J. S., Han, X., Zaharia, L. I., Abrams, S. R. and Prithiviraj, B. 2012.** Regulation of phytohormone biosynthesis and accumulation in *Arabidopsis* following treatment with commercial extract from the marine macroalga *Ascophyllum nodosum*. J. Plant Growth Regul. [Online] Available: http://link.springer.com/article/10.1007%2Fs00344-012-9301-9 [Oct. 2012].
**Zak, J. C., Willig, M. R., Moorhead, D. L. and Wildman, H. G. 1994.** Functional diversity of microbial communities: A quantitative approach. Soil Biol. Biochem. **26**: 1101–1108.
**Zhang, X. and Ervin, E. H. 2004.** Cytokinin-containing seaweed and humic acid extracts with creeping bentgrass leaf cytokinins and drought tolerance. Crop. Sci. **44**: 1–10.
**Zhang, X. and Ervin, E. H. 2008.** Impact of seaweed extract-based cytokinins and zeatin riboside on creeping bentgrass heat tolerance. Crop. Sci. **48**: 364–370.
**Zhang, X., Ervin, E. H. and Schmidt, R. E. 2003.** Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. Crop Sci. **43**: 952–956.
**Zhang, X., Yamane, H. and Chapman, D. J. 1993.** The phytohormone profile of the red alga *Porphyra perforata*. Bot. Mar. **36**: 257–266.