Effect of *Ascophyllum* extract application on plant growth, fruit yield and soil microbial communities of strawberry

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[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 28 November 2011, accepted 6 August 2012.*

Alam, M. Z., Braun, G., Norrie, J. and Hodges, D. M. 2013. **Effect of *Ascophyllum* extract application on plant growth, fruit yield and soil microbial communities of strawberry**. Can. J. Plant Sci. **93**: 23–36. Plant growth and associated soil microbials were examined in several strawberry cultivars following treatment with extracts from the marine algae *Ascophyllum nodosum* [soluble *Ascophyllum* extract powder (SAEP)]. Greenhouse and field experiments were established over plots of Albion, Camarosa, Chandler, and Festival strawberries from 2009 to 2011. Soluble *Ascophyllum* extract powder was applied once or twice per week, or once per 2 wk at rates of 0 (control), 1, 2 or 4 g L^{-1} over approximately 8 wk. Subsequent rooting studies examined weekly applications of SAEP at 0, 0.2, 0.4, 1 or 2 g L^{-1}. Results indicate that maximum plant and berry productivities were found at 1 and 2 g SAEP L^{-1} in both field and greenhouse. Chandler was the cultivar most responsive to SAEP application, while Albion was the least responsive. Soluble *Ascophyllum* extract powder increased colony counts in greenhouse and field soil samples with maximum colony counts at 4 g L^{-1} SAEP in the greenhouse, and 1 and 2 g L^{-1} SAEP in the field. Metabolic activities of soil microbes were found to increase following SAEP applications. Using the Biolog microbial analysis system, maximum average well colour development (AWCD), substrate diversity (H), substrate evenness (E), and substrate richness (S) responses were found at 4 g L^{-1} SAEP in the greenhouse. However, in field trials, AWCD, H, E, and S responses to extract treatment showed successive increases at 1 and 2 g L^{-1} SAEP, but reduced effect at 4 g L^{-1}. Soluble *Ascophyllum* extract powder treatment showed highest respiration rates between 0.10 and 0.40 g per week per plant while in vitro soil treatments with 4 g L^{-1} SAEP reduced microbial respiration. This study suggests that SAEP applications increased strawberry root and shoot growth, berry yield and rhizosphere microbial diversity and physiological activity.

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

Alam, M. Z., Braun, G., Norrie, J. et Hodges, D. M. 2013. **L'application d'un extrait d'*Ascophyllum* et son incidence sur la croissance et le rendement du fraisier ainsi que sur la microflore du sol**. Can. J. Plant Sci. **93**: 23–36. Les auteurs se sont intéressés à la croissance des plantes et à la microflore du sol après l'application d'un extrait de l'algue marine *Ascophyllum nodosum* (SAEP) à plusieurs cultivars de fraisier. Les essais en serre et au champ se sont déroulés de 2009 à 2011, sur des parcelles de fraisiers Albion, Camarosa, Chandler et Festival. Le SAEP a été appliqué une ou deux fois par semaine, ou une fois bimensuellement, à raison de 0 (témoin), 1, 2 ou 4 g L^{-1} pendant environ huit semaines. Lors d'études subséquentes sur l'enracinement, on a appliqué hebdomadairement 0, 0,2, 0,4, 1 ou 2 g L^{-1} de SAEP aux plantes. Les résultats indiquent une croissance maximale et un rendement fruitier optimal avec l'application de 1 et 2 g de SAEP par litre, au champ comme en serre. Chandler réagit le mieux à l'application de SAEP, tandis qu'Albion y est le moins sensible. Le SAEP augmente le nombre de colonies dans les échantillons recueillis au champ et en serre, le maximum survenant à 4 g de SAEP par litre en serre, et à 1 et 2 g de SAEP par litre au champ. Le métabolisme des microorganismes du sol s'accroît après l'application de SAEP. Quand on recourt au système d'analyse bactérien Biolog, la valeur moyenne la plus élevée pour la coloration des puits, la diversité du substrat, l'uniformité du substrat et la richesse du substrat est relevée au taux de 4 g de SAEP par litre, en serre. Lors des essais en pleine terre cependant, les mêmes paramètres augmentent successivement avec l'application de 1 et de 2 g de SAEP par litre, avant de diminuer lorsqu'on passe au taux de 4 g de SAEP par litre. Le traitement avec le SAEP entraîne le taux de respiration le plus intense entre 0,10 et 0,40 g par semaine par plante, alors que traiter le sol in vitro avec 4 g de SAEP par litre réduit la respiration microbienne. Les résultats de l'étude laissent croire que l'application de SAEP favorise la croissance des racines et des pousses de fraisier, le rendement fruitier ainsi que la diversité et l'activité physiologique de la rhizosphère bactérienne.

Mots clés: Extrait d'*Ascophyllum*, fraisier, microflore tellurique, profil Biolog, respiration du sol.

Abbreviations: **AWCD**, average well colour development; **E**, substrate evenness; **H**, substrate diversity; **PAF**, *Pseudomonas* Agar F; **RBCC**, Rose Bengal Media; **S**, substrate richness; **SAEP**, soluble *Ascophyllum* extract powder; **SDW**, sterilized deionized water; **TSA**, tryptic soy agar

The use of biostimulants may help improve current cropping system protocols. Marine bioactive substances extracted from marine algae (commonly known as seaweeds) are widely used in agricultural and horticultural crops and have shown many benefits in yield and quality (Blunden 1991; Crouch and Van Staden 1994; Khan et al. 2009).

Seaweeds have been used in crop production for centuries, especially in coastal areas. Seaweed extracts have also been used for decades, but have become more widely accepted over the past 10–15 yr, particularly in organic agriculture (Craigie 2011). Seaweed is beneficial to both soils and plants. However, in its raw form, seaweed must be broken down, or decomposed, before its benefits are available (Verkleij 1992). Seaweed extracts in either liquid or water-soluble powder form provide a readily available source of nutrients and organic compounds. Of course, characteristics of commercial extracts are dependent on seaweed species and the methods of preparation. Commercial and academic reports make claims such as 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 (Khan et al. 2009; Ross et al. 2011).

The productivity of agricultural systems is strongly influenced by soil microbes (Killham 1994; Singh et al. 2011). Soil microbes play an intrinsic role in residue decomposition and nutrient recycling. Effects of seaweed extract treatments on soil microbes may lead to improved plant performance. A recent study showed that extracts from *Euchema denticulatum* exhibit antibacterial activity (Al-Haj et al. 2009) while Jayaraj et al. (2008, 2011) and Ross et al. (2011) demonstrated that *Aschophylum nodosum* extract induced a number of disease resistance factors in crop plants and a subsequent reduction in disease severity. In contrast, Giotis et al. (2009) reported no effects of commercial seaweed extract (Marinure) on soil-borne plant pathogenic fungi (*Pyrenochaeta lycopersici* and *Verticillium albo-atrum*). Understanding microbial community structure shifts following implementation of various land use and management systems, such as the use of seaweed extracts, may lead to improved ''best management practices'' for agroecosystems.

The recent development of culture-independent molecular techniques has helped create a better understanding of the soil microbial community (Garland and Mills 1991; Smit et al. 2001). Using the Biolog microplate identification systems (Garland and Mills 1991), it is possible to obtain an assessment of functional differences in microbial communities from a variety of soil and water samples based on sole-source carbon utilization patterns of bacterial communities (Garland and Mills 1991; Zak et al. 1994). Although the exact numbers and taxonomic identities of the bacterial species responsible for the Biolog reactions remain to be determined, patterns of functional diversity within and among communities provide meaningful insight into soil communities where little understanding currently exists.

This study investigated the effects of soluble *Ascophyllum nodosum* extract powder (SAEP) treatments on growth, yield and microbial dynamics for four varieties of strawberry under greenhouse and field conditions.

MATERIALS AND METHODS

Plants and Seaweed Extract

Three of the strawberry varieties (Chandler, Festival and Camarosa) were purchased from the Lassen Canyon Nursery, California, US, and Albion was sourced from C.O. Keddy Nursery Inc., Nova Scotia, Canada. A commercially available (Acadian^{TM}), 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.

Greenhouse Study

To investigate the effects of SAEP on strawberry root growth, previously frozen daughter plants (refer to young plantlets held at $-2^{\circ}C$ as part of the vernalization/storage process; plantlets were held in this state until transplantation) of the four strawberry varieties (*Fragaria ananassa* 'Albion' 'Camarosa', 'Chandler', and 'Festival') were potted (on 2010 Jun. 08) in 12.5 cm diam. $\times$ 12.5 cm deep (1 L, $5'' \times 5''$ standard) plastic pots filled with soil-less growth medium PRO-MIX 'BX' (Premier Horticulture, Rivière-du-Loup, QC) consisting of 82.5% peat moss, 12.5% perlite and 5% vermiculite (by volume).

Prior to planting, roots were trimmed to 6.0 cm and uniformly thinned (if needed) for all treatments. Pots were spaced at 25×25 cm in a grid system on 90-cm high benches in the greenhouse. There were four replications and three plants per treatment. Average day and night greenhouse temperature ranged between 17 and $30^{\circ}C$ during the growth period (2010 Jun. 08 to Aug. 04). Light levels (500–1000 $\mu mol\ s^{-1}\ m^{-2}$) and humidity (60–90%) were under ambient summer conditions.

Fertilization began the next day following potting. Starting on 2010 Jun. 09, 200 mL of 15–15–18 soluble fertilizer (Plant Products Co. Ltd., Brampton, ON) was applied at the rate of 2 g L^{-1} once every 2 wk (on Wednesday) to each pot. During the following week, 200 mL of $Ca(NO_3)_2$ (greenhouse grade, 15.5% N and 19% Ca. Plant Products Co. Ltd., Brampton, ON) was applied at 2 g L^{-1} once every 2 wk for 6 wk. This alternate application of 15–15–18 soluble fertilizer and $Ca(NO_3)_2$ continued until the end of the experiment

(8 wk). Plants were irrigated with tap water (pH 7.31, EC 0.46 dS m^{-1}) as necessary.

Starting on 2010 Jun. 10, 100 mL of 0.2, 0.4, 1.0 or 2.0 g L^{-1} SAEP was applied manually to each pot weekly. Soluble *Ascophyllum* extract powder solutions were prepared by dissolving powdered *Ascophyllum* extract in tap water. Control treatments received 100 mL of water. Plants were harvested at 2, 4, 6 and 8 wk after treatment started and roots and shoots were separated at the soil level. Roots were washed and scanned (EPSON Expression 10000 XL. Model: J181A, SEIKO EPSON Corp., 80 Harashinden, Hirooka Shojiri-Shi, Nagano-Ken 399-0785, Japan) for root variables [total root length, total root surface area, total root volume, root diameters (<0.5 mm, 0.5–<1.0 mm, 1–<1.5 mm, 1.5–<2.0 mm, 2–<2.5 mm and >2.5 mm)].

To investigate effects of SAEP on soil microbes, bulk soil (to 15 cm depth) was collected (during April 2009) from a homogeneous field located near Agriculture and Agri-Food Canada, Kentville, NS (lat. 45°4′39″, long. 64°9′45″). The sandy loam soil had an average particle size distribution of 87.7% sand, 8.9% silt, and 3.5% clay. On 2009 May 13, previously frozen daughter plants of the above mentioned four strawberry varieties were potted in similar size plastic pots filled with 80% field soil and 20% perlite (by volume). Prior to planting, roots were trimmed and thinned as usual. Pots were spaced in the greenhouse with average day and night temperatures as previously noted.

Fertilization began 1 wk after potting. Fertilizer grades, application rates and irrigation protocols were the same as in the rooting study. However, the SAEP treatments consisted of 100 mL of 1, 2 or 4 g L^{-1} SAEP solution applied manually to each pot once weekly, twice weekly or once every 2 wk. Soluble *Ascophyllum* extract powder solutions were prepared by dissolving powdered *Ascophyllum* extract in tap water as mentioned above. Control treatments received 100 mL of deionised water.

Field Plot Study

Two late-planting field plot studies (2009 Aug. 12 to Sep. 28 and 2010 Aug. 12 to Sep. 28) and one early planting field plot study (2010 May 19 to 2011 Aug. 15) were conducted at Agriculture and Agri-Food Canada's Sheffield Mills Research Farm (Sheffield Mills, Nova Scotia. lat. 45°38′, long. 64°30′). The silt loam soil is an Orthic Humo-Ferric Podsol with a mean particle size distribution of 87.7% sand, 8.9% silt, and 3.5% clay (Langille 1986). The area is characterized by a cool humid temperate climate with mean annual temperature of 6.9°C and precipitation of about 1116 mm per year. Frozen daughter plants of the same four strawberry varieties (Chandler, Festival, Camarosa, and Albion) were planted in rows spaced 45 cm apart (30 cm plant-to-plant). Before planting, roots were trimmed to 6.0 cm and thinned as before. For each treatment, there were four replications of three plants/replicate.

For late-planting studies, fertilization began 2 wk after planting. To each plant, 200 mL of $Ca(NO_3)_2$ (greenhouse grade, 15.5% N and 19% Ca. Plant Products Co. Ltd., Brampton, ON) was applied at 2 g L^{-1} once per week. During the following week, 200 mL of 15–15–18 soluble fertilizer (Plant Products Co. Ltd., Brampton, ON) at 2 g L^{-1} was applied weekly. Alternate applications of $Ca(NO_3)_2$ and 15–15–18 continued until the end of the studies (about 7 wk). Plants were irrigated with tap water as necessary.

Ascophyllum extract treatments began 1 wk after planting. Each plant received 100 mL of either1, 2 or 4 g L^{-1} SAEP twice weekly, once weekly, or once per 2 wk. Soluble *Ascophyllum* extract powder solutions were prepared by dissolving powdered *Ascophyllum* extract in tap water. Control treatments received 100 mL of tap water.

For the early planting study, we followed the same protocols for irrigation, fertilizer and SAEP treatment applications for 7 wk. Then the plants were over wintered and the same irrigation, fertilizer and SAEP treatment application protocols were followed in the following spring until harvest of berries (2011 Jul. 07) for yield and yield component studies.

Soil Sample Collection

After 6 wk in the greenhouse, strawberry plants were removed from containers and the soil and root mass was placed inside a plastic bag, which was then shaken gently to collect 200 g of rhizosphere soil (soil around the roots). At the end of the late planting field plot studies (2009 and 2010) and early planting (2010 only, before over wintering), composite (three cores from each of three replicate plants per treatment) surface (0 to 10 cm depth) root-zone soil samples were collected. Soil samples were sieved through a 2-mm sieve to separate plant debris, pebbles, etc. Bacterial colony counts, microbial respiration and Biolog assays were performed immediately on fresh soil for both greenhouse and field samples. While data on growth response to SAEP applications were collected for all varieties, data on soil microbes was collected only for cv. Festival.

Soil Microbial Colony Counts in Response to SAEP Application

The number of bacteria in soil samples were determined by transferring 2 g of the rhizosphere soil into 100 mL bottles containing a 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 and allowed 30 s for settling of the heavy particles. Then 1.0 mL of the suspension was transferred to a fresh bottle containing 99 mL of sterile saline water, shaken for ~30 s and then 492 μL 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 (Gilchrist et al. 1973) plater (Advanced Instruments Inc. Two Technology Way, Norwood, MA). Plates were incubated at $27 \pm 1^{\circ}C$ in the dark and bacterial colonies were counted under a microscope at 24 h intervals until no new colonies appeared. The number of bacterial colony forming units per gram of soil was calculated using the dilution factor:

$$Y = (b \div 0.492) \times 100 \times 50$$

where Y is the number of bacterial colony forming units per gram of rhizosphere soil and b is the number of bacterial colonies found in each Petri dish.

Biolog Community Level Physiological Profile of SAEP-treated Strawberry

The bacterial community level physiological profile was determined with Biolog ECO® 96-microwell plates with 31 carbon sources and a control replicated three times per plate. The 2 g soil suspension prepared for colony counts described above was allowed to settle for 30 s after shaking and then 1 mL was 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 the wells of the ECO plate and incubated at $27 \pm 1^{\circ}C$ in the dark. Colour development was measured at 590 nm and 750 nm after 24, 48, 72, and 96 h incubation with an automated plate reader (Biolog Microstation, Biolog Inc. 21124 Cobat Blvd. Hayward, CA) and data were collected using Microlog 4.01 software (Biolog Inc. 21124 Cobat Blvd. Hayward, CA).

Average well colour development (AWCD) was calculated by the method of Garland (1996). Briefly, the absorbance value at 590 nm wavelength of the control well was subtracted from all other wells on the plate. To correct for weak false-positive readings, 0.250 absorbance units was subtracted from all well values. Wells with negative values were assigned a value of zero. To compensate for differences in inoculum concentration (normalization) all 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 95 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 (Zak et al. 1994):

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

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.

Respiration of SAEP-treated Soil

For soil respiration studies, SAEP-treated 2010 field soil samples were collected depending upon amount of SAEP treatment received by each plant per week. Fifty grams of sieved (2 mm) soil for each treatment including control [blank, 5 mL of sterilized deionized water (SDW) + no soil] was placed into 4-L glass jars on weighing boats. The effect of SAEP application in vitro was measured applying 5 mL of SAEP solution at rates of 0, 1, 2 or 4 g L^{-1} SAEP per 50 g of soil. This rate of application approximated the effect of applying SAEP to 1-L pots of field soil in the greenhouse study. An additional control with 5 mL of SAEP with no soil was also used in this study. The treatments were as follows: (i) Blank (5 mL SDW + no soil); (ii) Control (5 mL of SAEP + no soil); (iii) SAEP 0 (50 g soil + 5 mL SDW); (iv) SAEP 1 (50 g soil + 5 mL of 1 g L^{-1} SAEP); (v) SAEP 2 (50 g soil + 5 mL of 2 g L^{-1} SAEP); and (vi) SAEP 4 (50 g soil + 5 mL of 4 g L^{-1} SAEP). Ten millilitres of SDW water was used to wet a piece of chromatography 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 were measured every 24 h for 4–5 d as a percent CO_2 using an ICA 41, 42, 43 modular gas analyzer (International Controlled Atmosphere Ltd., Kent, UK). Rate of CO_2 production was expressed as milligrams of CO_2 kilogram of dry soil per day.

Statistical Analysis

Greenhouse and field experiments used a three-factor (strawberry variety, SAEP rate and SAEP application timing) randomized complete block design with four replications and three plants per replicate in the greenhouse and four replications and three plants per replicate in the field. Data were analyzed following standard statistical procedure 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 significance test (LSD).

RESULTS

Root Growth

Ascophyllum extract application showed significant effects on strawberry total root length, total root surface area, total root volume and number of roots (<0.5 mm and 1–1.5 mm diam.) per plant in the greenhouse. However, there were no significant differences between harvest dates (2, 4, 6 and 8 wk) and cultivars. In the absence of treatment differences between cultivars and harvest dates, results were averaged over all cultivars.

A significant increase in root length was observed when plants were treated with 0.2 and 0.4 g L^{-1} SAEP but not for 1 and 2 g L^{-1} SAEP-treated plants (Fig. 1a). Total root surface area was significantly greater under 0.4 and 1.0 g L^{-1} SAEP treatments but no differences were observed between the control and 0.2 and 2 g L^{-1} SAEP-treated plants (Fig. 1b). Total root volume was also significantly increased under 0.4 and 1.0 g L^{-1} SAEP treatments compared with the control. However, 0.2 and 0.4 g L^{-1} treatments were similar with no differences between the control and either 0.2 and 2 g L^{-1} SAEP treatments (Fig. 1c). Irrespective of rates, SAEP treatments increased the number of fine roots (< 0.5 mm diam.) (Fig. 1d) up to 1 g L^{-1}. The 2 g L^{-1} SAEP treatment resulted in a reduced number of fine roots. A significant increase in 1–1.5 mm diameter roots was also observed when plants were treated with at 1.0 g L^{-1} (data not presented).

Shoot Growth

Combined analysis of 2009 and 2010 data showed that *Ascophyllum* extract applications had a profound effect on strawberry shoot growth under field conditions. As interactions between cultivars and SAEP rates were not significant and there were no significant differences between application timing treatments for the measured variables, results were averaged for leaf area and shoot fresh and dry weights. The results showed that all SAEP treatments irrespective of rates, induced a significant and similar increases ($P < 0.001$) in leaf area (Fig. 2a) and shoot dry weights (Fig. 2b) of strawberry plants compared to water-treated controls.

Berry Productivity

Ascophyllum extract application had significant effects on berry number and berry yield per plant (in field). However, the response varied between cultivars. Berry size was not influenced by *Ascophyllum* extract treatment for any of the cultivars (data not shown). No significant differences were found between application timings. Therefore, results for timings of once/week, twice/week, and once every 2 wk were averaged.

Soluble *Ascophyllum* extract powder treatment had no effects on cv. Albion. However, for the other three cultivars; 1 g L^{-1} SAEP treatment showed significant ($P < 0.001$) increases in berry number and berry yield per plant (Figs. 3a and b). Treatment of plants with 2 g L^{-1} SAEP had either similar effects (cv. Camarosa and Festival) as 1 g L^{-1} treatment, or led to reduced berry number and berry yield (cv. Chandler) when compared with 1 g L^{-1} SAEP. Treatment of plants with 4 g L^{-1} SAEP had comparable berry number or berry yield per plant as control (except for berry yield of cv. Chandler). Treatments, however, led to a reduced berry number and berry yield per plant for those three berry cultivars when compared with plants treated with 1 g L^{-1} SAEP (Figs. 3a and b).

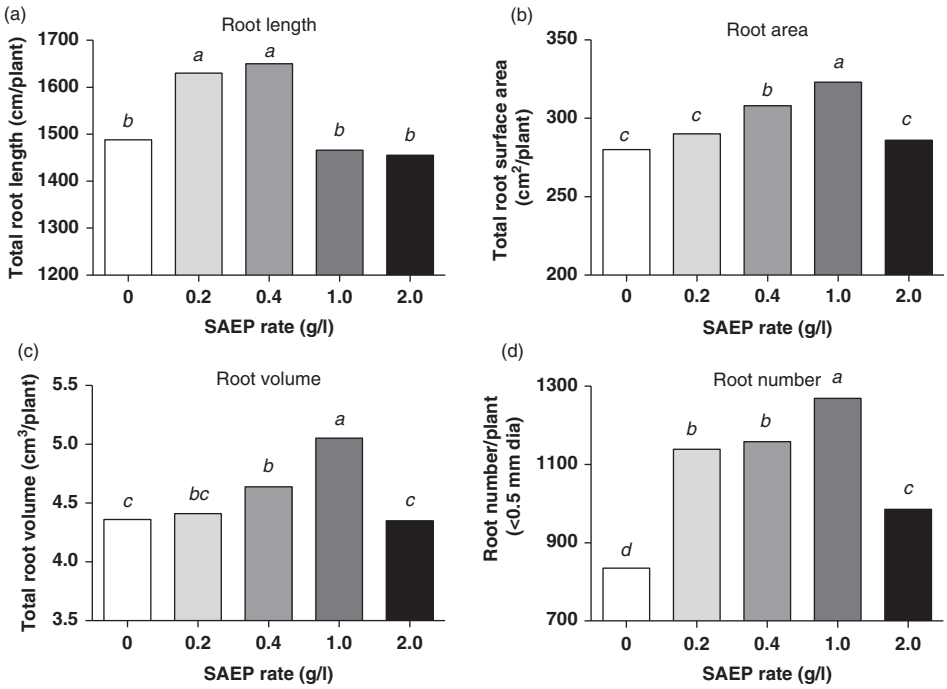

Fig. 1. Effect of soluble *Ascophyllum* extract powder on total root length (a), total root surface area (b), total root volume (c), and fine root (<0.5 mm diam.) numbers (d) per strawberry plant in the greenhouse.

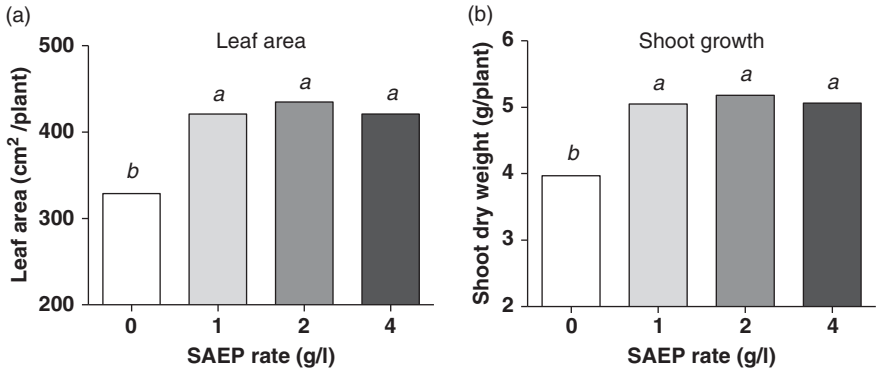

Fig. 2. Effect of soluble *Ascophyllum* extract powder on leaf area (a) and shoot dry weight (b) per strawberry plant in the in the field (combined data of late planting 2009 and 2010).

Colony Counts

Microbial colony (PAF, TSA and RBCC) development varied in response to different SAEP rates and application timings under greenhouse and field conditions. On average, colony counts were about four- to sevenfold higher in the greenhouse than in the field during 2009 (Figs. 4a and b) and 2010, respectively (Figs. 4a, c and d). At 4 g L^{-1} SAEP, the number of colonies was significantly higher under greenhouse conditions compared with water-treated controls, irrespective of application timings (Fig. 4 a). However, at 1 and 2 g L^{-1} SAEP, colony numbers did not increase except at 2 g L^{-1} once/week and at 1 g L^{-1} once/2 wk treatments.

Under field conditions during early and late plantings 2009 and 2010 (Figs. 4b, c and d), colony counts were highly dependent upon SAEP application rate and timing. At an application frequency of twice/week, 1, 2 and 4 g L^{-1} SAEP-treated soil exhibited higher colony counts than water-treated soil, with the highest colony counts at 1 g L^{-1}. Soluble *Ascophyllum* extract powder application rates of 1, 2 and 4 g L^{-1} once/week also significantly increased colony counts over those of the water-treated controls with highest colony counts at 2 g L^{-1}. At an application frequency of once every 2 wk, only 2 and 4 g L^{-1} rates showed higher colony counts than the control, with 4 g L^{-1} treated soil having significantly higher counts than 2 g L^{-1} treated soil. SAEP application frequency of twice every week and once every 2 wk had similar and lower colony counts compared with once every week.

For the early planting field trial in 2010, SAEP rates of 1 and 2 g L^{-1} applied twice/week had similar colony counts compared with water alone, while the 4 g L^{-1} rate significantly reduced colony counts (Fig. 4c). At once/week and once/two weeks, 1, 2 and 4 g L^{-1} had similar or greater colony counts compared with water controls (Fig. 4c). Slowing the frequency of SAEP application to once/week and once/2 wk showed greater and similar colony counts compared with twice per week applications.

The late 2010 field trial colony counts were similar over all rates of SAEP, including water control at an application frequency of twice per week (Fig. 4d). At once/week application, however, 1 and 2 g L^{-1} showed higher colony counts compared with control. Applications at 4 g L^{-1} was comparable with the water-treated control. Once per 2 wk frequencies showed significantly

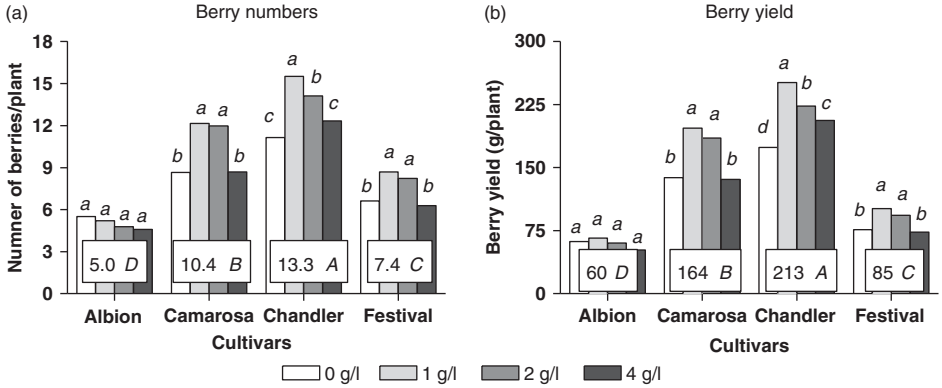

Fig. 3. Effect of soluble *Ascophyllum* extract powder on number of berries (a) and berry yield (b) per strawberry plant in the in the field. Values in the boxes indicate cultivar averages.

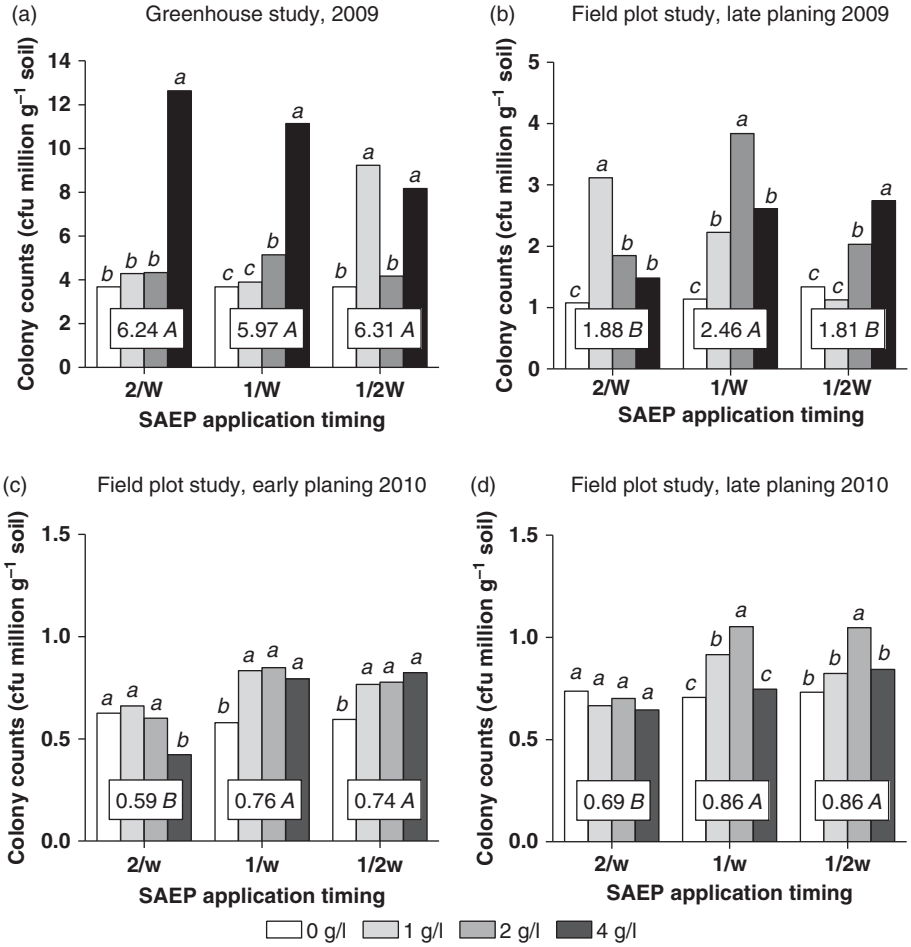

Fig. 4. Effect of soluble *Ascophyllum* extract powder on microbial colony counts in greenhouse, 2009 (a) and in field, late planting 2009 (b), early planting 2010 (c) and late planting 2010 (d). Values in the boxes indicate cultivar averages.

greater colony counts at 2 g L^{-1} compared with other treatments, while 1 and 4 g L^{-1} were comparable with water-treated controls (Fig. 4d). Application frequencies of once per week and once every 2 wk had similar and greater colony counts compared with twice/week (Figs. 4c and d).

Biolog Analysis

The carbon substrate metabolic activities of the soil microbes were characterized in greenhouse and field plot studies using AWCD, substrate diversity (H), evenness (E), and substrate richness (S) measured at 24, 48, 72, and 96 h incubation periods. Only data with statistically significant differences are presented here. Results for application frequencies of twice/week, once/week, and once every 2 wk were averaged for AWCD, H, E, and S due to a lack of significant differences between application frequencies.

The AWCD, H, E, and S of all carbon sources generally showed similar and linear responses to different SAEP rates in the greenhouse with highest values at 4 g L^{-1} (Figs. 5a, b, c and d). For AWCD, H and E variables, all SAEP treatments led to increases in comparison with water-treated soils, but there were no significant differences between soils treated with 1 and 2 g L^{-1} SAEP. However, for S, there was no significant difference between water-treated soil and the 1 or 2 g L^{-1} treatments.

Results were somewhat different in field trials (Figs. 6–8a, b, c and d). For the late-planted field trial in 2009, the AWCD, H, E, and S variables at the 1 and 2 g L^{-1} SAEP rates gave significantly higher results except for the AWCD at 2 g L^{-1} SAEP-treatment. Soil treated with the 4 g L^{-1} SAEP treatments showed no differences (Figs. 6a, b, c and d). Similar trends were observed for the early-planted strawberries in 2010 (Figs. 7a, b, c and d). At 1 and 2 g L^{-1} rates of SAEP, AWCD, H, E, and S variables had greater values. Soil treated with 4 g L^{-1} SAEP though provided similar results for AWCD and S variables, resulted greater values for H and E variables. For the late plantings in 2010 (Figs. 8a, b, c and d), AWCD results were greater for 2 g L^{-1} SAEP treatments, while 1 and 4 g L^{-1} SAEP treatments

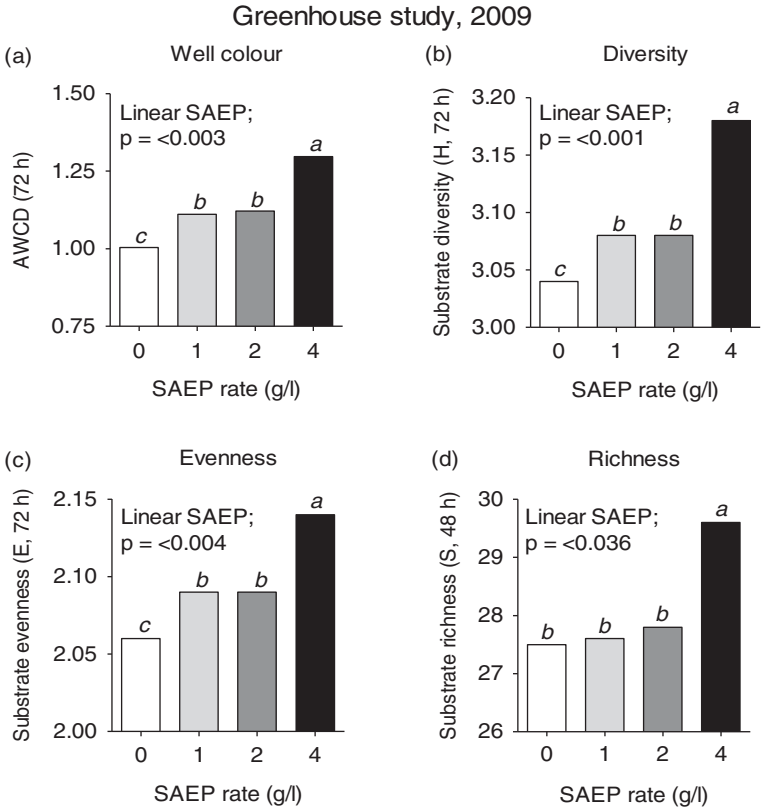

Fig. 5. Effect of soluble *Ascophyllum* extract powder on average well colour development [AWCD, (a)], substrate diversity [H, (b)], substrate evenness [E, (c)], and substrate richness [S (d)] in the greenhouse, 2009.

provided values similar to the water-treated control. For H, however, 1 g L^{-1} treatment gave higher values. Soluble *Ascophyllum* extract powder rates of 2 and 4 g L^{-1} were comparable with water-treated control and 1 g L^{-1}. For E and S variables, 1, 2 and 4 g L^{-1} SAEP treatments gave similar and higher values compared with controls, respectively.

Soil Respiration

For early plantings in 2010, soils receiving 0.2, 0.4 and 0.8 g of SAEP treatments per week had higher respiration with the highest respiration measurement occurring at 0.2 g w^{-1} followed by 0.40 and 0.80 g w^{-1} (Fig. 9a). Similar results were observed for the late planting field trial in 2010 (Fig. 9b). Contrast analysis showed that SAEP treatments irrespective of rates had higher soil respiration with highest respiration occurring at 0.10 g w^{-1} and 0.20 g w^{-1}. Soluble *Ascophyllum* extract powder rates of 0.05 and 0.40 g w^{-1} had similar and greater respiration compared to 0.80 g w^{-1}. In vitro SAEP treatment of freshly collected field soil showed that treatment with 4 g L^{-1} SAEP gave markedly lower soil respiration compared with all other treatments (Fig. 9c). Treatment with 1 and 2 g L^{-1} SAEP had similar soil respirations as the controls.

DISCUSSION

This study investigated effects of soluble *Ascophyllum* extract powder application on root and shoot characteristics, fruit yield and rhizospheral microbial communities in strawberry. Our results show that *Ascophyllum* extract application increased total root length, root surface area, root volume per plant and number of <0.5 and 1–1.5 mm diameter roots of strawberry plants in the greenhouse. Field results demonstrate that SAEP treatment increased leaf area and shoot fresh/dry weights of strawberry irrespective of rates and application timings. However, maximum number of berry and berry yields were recorded when plants were treated with 1 or 2 g L^{-1} SAEP in all tested cultivars except Albion, irrespective of application timing. *Ascophyllum* extract application also increased total soil microbial colony counts and total microbial physiological activity in soil samples taken from the greenhouse and field and soil respiration in the field. However, microbial colony counts and activities varied between the two growing environments.

Bioactive substances extracted from marine algae have been reported to have many beneficial effects in terms of enhancement of root and shoot growth, yield, and quality in many agricultural and horticultural crops (Abdel-Hafeez 2005; Zhang and Ervin 2008; Fan et al.

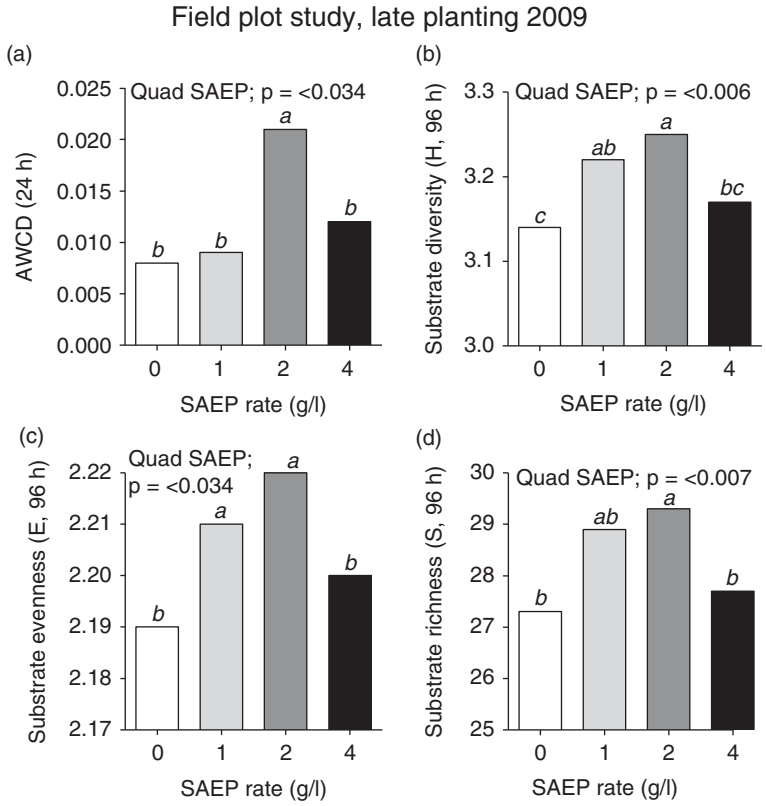

Fig. 6. Effect of soluble *Ascophyllum* extract powder on average well colour development [AWCD, (a)], substrate diversity [H, (b)], substrate evenness [E, (c)], and substrate richness [S (d)] in the field, late planting, 2009.

2011). Results of our studies also confirm these observations. Seaweed extracts contain several plant growth regulatory substances such as betaines, polyamines and oligosaccharides (Blunden et al. 1986), amino acids and vitamins (Mooney and van Staden 1986). *Ascophyllum nodosum* extracts also contain low levels of cytokinins and auxins as well as moderate levels of other growth stimulants and nutrients (Mooney and van Staden 1986; Crouch et al. 1992). These compounds have been shown to positively affect growth of plant shoot and root tissue (Zhang et al. 2003; Zhang and Ervin 2004) and have been reported to stimulate the growth and yield of plants (Khan et al. 2009). Seaweed extract also contains major and minor elemental nutrients. The *Ascophyllum* extract (Acadian Seaplants Ltd. SAEP Batch # 5221) used in these particular studies contained about 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. However, as SAEP was diluted to 1, 2 and 4 g L^{-1} prior to application (particularly for shoot and berry productivity studies), these nutrients were thus applied at low concentrations relative to the crop requirements and most likely would not significantly impact on growth relative to the regular fertility program.

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. In our studies, we also observed positive effects of SAEP on root length, root surface area, root volume and root numbers ($<$0.5 and 1–1.5 mm diam.) in the greenhouse at rates between 0.2 and 1 g L^{-1}. Soluble *Ascophyllum* extract powder at 2 g L^{-1} led to a negative impact on root characteristics. Growth responses to *Ascophyllum* extracts have been shown to be similar to those induced by plant growth regulators such as a cytokinins and auxins. In general, cytokinins are known to stimulate cell division and differentiation. In turn, root elongation and expansion may be associated with endogenous gibberellin production (Alam and Chong 2006). Nevertheless, the more vigorous root system associated with seaweed extract application may provide a larger surface area for nutrient and water uptake and allow for improved mineral nutrition and growth. As a consequence of improved nutrition and the presence of plant growth-promoting substances mentioned above in seaweed extracts, seaweed extract-treated plants often have

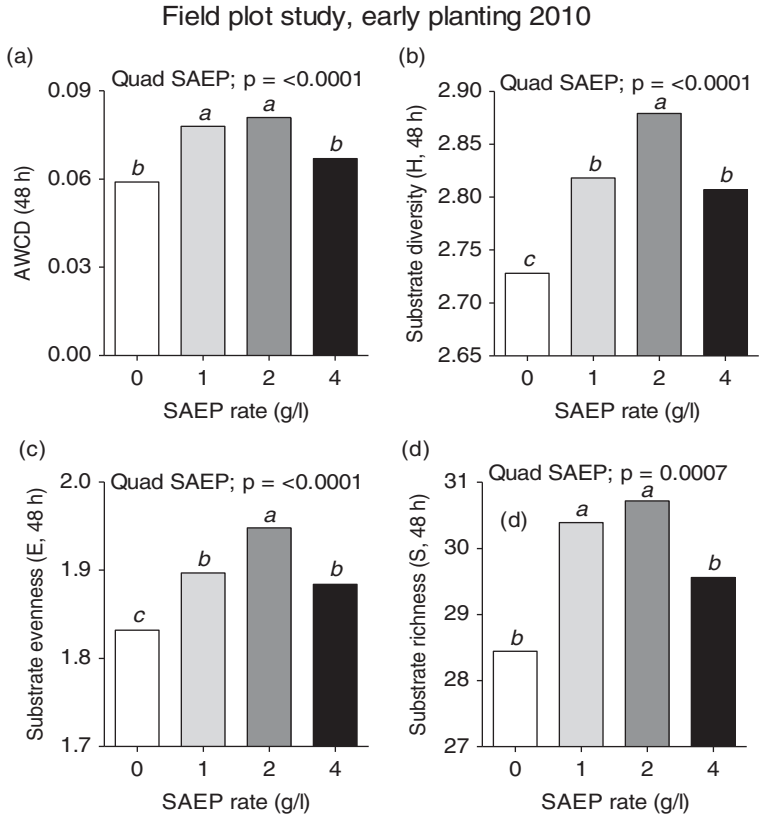

Fig. 7. Effect of soluble *Ascophyllum* extract powder on average well colour development [AWCD, (a)], substrate diversity [H, (b)], substrate evenness [E, (c)], and substrate richness [S (d)] in the field, early planning, 2010.

more vigorous growth and yield as measured by an increase in leaf area, root and shoot fresh/dry weights, berry numbers and berry yield in these studies. In this study, cultivar differences in response to *Ascophyllum* extract application in terms of strawberry productivity (Chandler being the most responsive followed by Camarosa and Festival) may be related to inherent genetic characteristics of each cultivar. The lack of response of Albion may have been related to its everbearing nature. Our results suggested that maximum berry yields were achieved at SAEP rates between 1 and 2 g L^{-1}, and the increased berry yield was due to the increased number of berries per plant as *Ascophyllum* extract application had no effects on berry size.

Microorganisms are considered to be effective bioindicators due to their capacity to respond quickly to environmental changes (Avidano et al. 2005; Singh et al. 2011). Our results show that total colony counts were four- and sevenfold greater in the greenhouse than those in the field during 2009 and 2010, respectively. This difference is probably related to ambient temperature differences between the two growing conditions and soil sampling (rhizosphere soil vs. root-zone soil). Greenhouse day and night temperatures ranged between 17 and 30°C during the entire growth period (2009 May 13 to Jun. 24), while in the field, day and night temperature ranged between 11 and 25°C for the early planting study 2010 and between 1.8 and 24.2°C during the last 2 wk of plant growth for the late-planting studies during 2009 and 2010. Cooler soil temperatures in the field plot study may have reduced bacteria in soil relative to the warmer greenhouse environment. Differences in colony counts between the late-planted 2009 and 2010 early- and late-planted field experiments may be related to soil properties. Although the location of three field trials was the same over the years, three different plots were chosen to conduct each trial. Although individual microbes were not examined in detail, our data show that SAEP treatments at 1, 2 and 4 g L^{-1} increased bacterial populations that grew on PAF by 39, 29 and 15%, respectively. However, the numbers of fluorescent *Pseudomonad* species were too low to accurately quantify. It is well established that a large number of plant-beneficial bacteria belong to the *Pseudomonas* genus. Total culturable bacteria (TSA culture medium) increased by 4, 23 and 10%, respectively, in response to applications of 1, 2 and 4 g L^{-1} of SAEP. *Ascophyllum* extract treatment increased fungal populations by 4 and 8% at 1 and 2 g L^{-1}, respectively, and reduced these populations by 21% (data not presented) at 4 g L^{-1}. Spinelli et al. (2010) also reported Actiwave® (a seaweed extract product derived

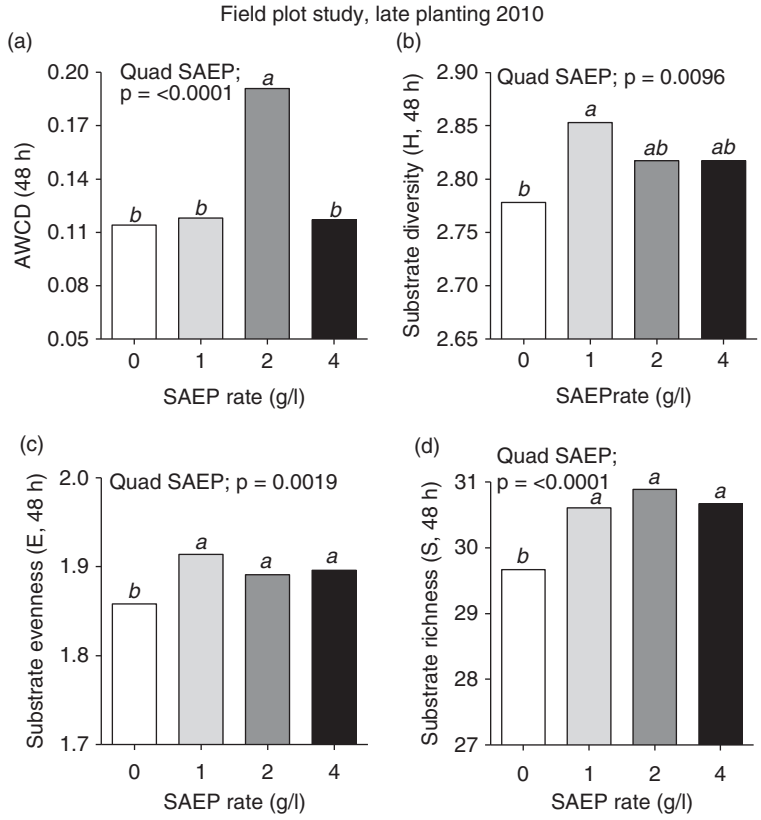

Fig. 8. Effect of soluble *Ascophyllum* extract powder on average well colour development [AWCD, (a)], substrate diversity [H, (b)], substrate evenness [E, (c)], and substrate richness [S (d)] in the field, late planning, 2010.

from *Ascophylum nodosum*) positively influenced root-associated microbial (*Pseudomonas* spp., *Bacillus subtilis* and related species and *Streptomyces* spp.) biocoenosis.

As the productivity of agricultural systems is known to depend heavily upon the functional processes of soil microbial communities (Killham 1994; Singh et al. 2011), we investigated soil microbial communities in response to SAEP application. Understanding the complex microbial community in the soil environment has proven to be a challenging task because of the vast diversity and enormity of the population (Sun et al. 2004). However, analyses of microbial metabolic activity, substrate diversity, richness, or evenness provide insight into whether the functional aspects of biodiversity differ among sites or among habitats within a site (Girvan et al. 2003). Average well colour development is an indicator of the extent of microbial metabolic activity. Substrate diversity (H) consists of species richness, the total number of species present, species evenness, and the distribution of species (Trevors 1998; Ovreas 2000). Substrate richness (S) is the number of different substrates that were utilized by the microbial community. Substrate evenness (E) measures the equitability of activities across all utilized substrates. The measurement of soil microbial physiological activity is being measured on mixtures of slow- and fast-growing bacteria. If fast-growing bacteria predominate, the population colour development in Biolog well plates is rapid and significant differences between treatments may appear after just 24 to 48 h. However, if slower-growing bacteria predominate the soil population it may take 72 or 96 h of growth before significant differences between treatments become apparent. In the greenhouse, the highest colony counts and microbial activities, measured in terms of AWCD, H, E, and S were following treatment with 4 g L^{-1} SAEP. In contrast in the field, the highest colony counts and AWCD, H, E, and S values were most often found following 1 and 2 g L^{-1} SAEP applications. However, reductions in colony numbers and AWCD, H, E, and S for the highest rates of SAEP application in the field may be related to suppressive effects of SAEP on microbes at higher rates. Suppressive effects of seaweed extracts on microbes have also been reported by others at higher concentrations. A seaweed extract (*Euchema denticulatum*) showed in vitro inhibitory effects on Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), although, Gram negative pathogens tested including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were found to be resistant (Al-Haj et al. 2009). Moreover, Sobolewski et al. (2007) reported that seaweed extract derivatives (Physpe 6 and Physpe 7)

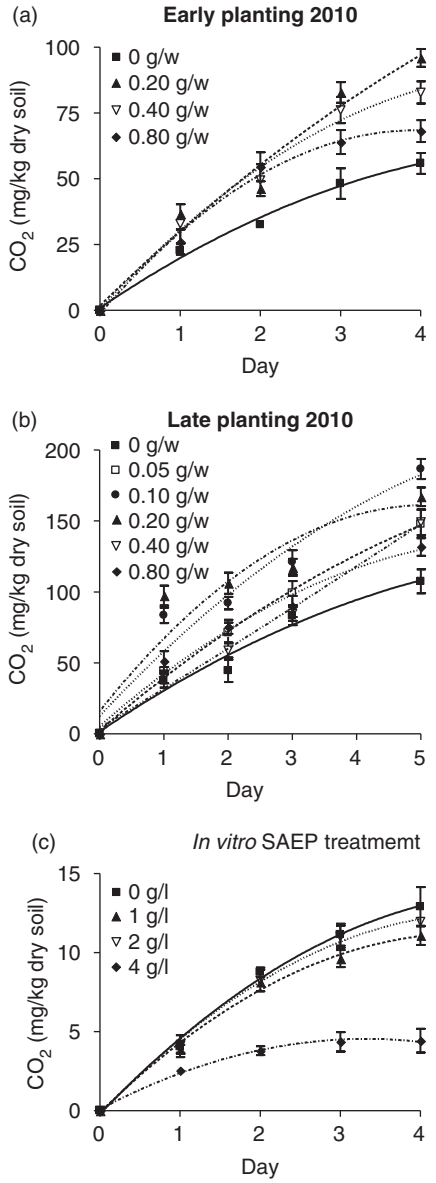

Fig. 9. Effect of soluble *Ascophyllum* extract powder on soil respiration when applied to field plots planted early spring 2010 (a), a late summer planting in 2010 (b) and in an in vitro assay on a composite field soil sample exposed to soluble *Ascophyllum* extract powder rates similar to field soil applications (c).

gave satisfactory control of bacterial speck (*Pseudomonas syringae* pv. *tomato*) and late blight (*Phytophthora infestans*) on tomato grown in the field and greenhouse.

Our soil respiration studies during 2010 (both early and late planting) in the field (Figs. 9 a and b) and more specifically, soil respiration in response to in vitro SAEP treatment (Fig. 9 c) clearly demonstrate that *Ascophyllum* extract has suppressive effects on microbes at higher rates under our study conditions. Nevertheless, these studies clearly demonstrate that microbial colony counts, metabolic activity, functional diversity and soil respiration increased in response to lower rates of SAEP (1 and 2 g L^{-1}).

In recent years, the use of seaweed extracts has gained in popularity due to their potential use in organic and sustainable agriculture (Craigie 2011) as a means to avoid excessive fertilizer applications and to improve mineral absorption. 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). In this study, increases in microbial colony counts were accompanied by increasing soil respiration and soil microbial physiological activity and diversity as the rate of SAEP application increased up to 4 g L^{-1} in the greenhouse and in the 1–2 g L^{-1} range in the field. Whether the increased microbial activities due to SAEP application contributed to the increased strawberry growth remains speculative. However, it is well established that during growth, plants interact intimately with soil microorganisms that influence above-ground ecosystems by contributing to soil fertility, plant nutrition and to overall plant health (Sun et al. 2004; Singh et al. 2011). According to Avis et al. (2008), the growth-promoting ability of bacteria from the genus *Pseudomonas* results from the synergistic effect of different physiological and ecological mechanisms including the solubilisation of P sources and/or regulation of plant growth hormones. Different *Pseudomonads* produce the root promoting hormone indole acetic acid (Glick et al. 1997) or interfere with its degradation (Leveau and Lindow 2005). On the other hand, other *Pseudomonads* prevent the synthesis of plant growth-inhibiting levels of ethylene in the roots (Penrose et al. 2001). The level of these hormones within the root system plays a crucial role in the growth-promoting ability of a microorganism (Avis et al. 2008).

This study focused on the effects of *Ascophyllum* extract application on strawberry plant productivity and soil microbial communities and showed clear and positive effects on root, shoot and berry productivity and direct stimulation of soil microbes related to *Ascophyllum* extract rate in the field and greenhouse. We hypothesize that the stimulation of plant growth, berry productivity and microbial activity found in these studies may have been due, in part, to the biostimulant components of the *Ascophyllum* extract. To the best of our knowledge, this is the first study of *Ascophyllum* extract effects on strawberries and soil microbes. Future studies will attempt to use techniques to better link SAEP-induced changes in bacterial and fungal populations to increased plant growth. Further work to pinpoint differences in full-cycle field and greenhouse-grown strawberries would help elucidate additional rate-response effects as well as variety-specific differences in plant growth.

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