



## Research Article

SPECIAL ISSUE: The Role of Below-Ground Processes  
in Mediating Plant InvasionsActivated carbon decreases invasive plant growth  
by mediating plant–microbe interactionsNicole E. Nolan<sup>1</sup>, Andrew Kulmatiski<sup>1\*</sup>, Karen H. Beard<sup>1</sup> and Jeanette M. Norton<sup>2</sup><sup>1</sup> Department of Wildland Resources and the Ecology Center, Utah Agricultural Experiment Station, Utah State University, Logan, UT 84322-5230, USA<sup>2</sup> Department of Plant, Soil, and Climate, Utah State University, Logan, UT 84322-4820, USA

Received: 29 July 2014; Accepted: 30 October 2014; Published: 10 November 2014

Associate Editor: Inderjit

Citation: Nolan NE, Kulmatiski A, Beard KH, Norton JM. 2015. Activated carbon decreases invasive plant growth by mediating plant–microbe interactions. *AoB PLANTS* 7: plu072; doi:10.1093/aobpla/plu072

**Abstract.** There is growing appreciation for the idea that plant–soil interactions (e.g. allelopathy and plant–microbe feedbacks) may explain the success of some non-native plants. Where this is the case, native plant restoration may require management tools that change plant–soil interactions. Activated carbon (AC) is one such potential tool. Previous research has shown the potential for high concentrations of AC to restore native plant growth to areas dominated by non-natives on a small scale (1 m × 1 m plots). Here we (i) test the efficacy of different AC concentrations at a larger scale (15 m × 15 m plots), (ii) measure microbial responses to AC treatment and (iii) use a greenhouse experiment to identify the primary mechanism, allelopathy versus microbial changes, through which AC impacts native and non-native plant growth. Three years after large-scale applications, AC treatments decreased non-native plant cover and increased the ratio of native to non-native species cover, particularly at concentrations >400 g m<sup>-2</sup>. Activated carbon similarly decreased non-native plant growth in the greenhouse. This effect, however, was only observed in live soils, suggesting that AC effects were microbially mediated and not caused by direct allelopathy. Bacterial community analysis of field soils indicated that AC increased the relative abundance of an unidentified bacterium and an Actinomycetales and decreased the relative abundance of a Flavobacterium, suggesting that these organisms may play a role in AC effects on plant growth. Results support the idea that manipulations of plant–microbe interactions may provide novel and effective ways of directing plant growth and community development (e.g. native plant restoration).

**Keywords:** Allelopathy; Archaea; bacteria; invasive species; native plants; plant–soil feedback; pyrosequencing; restoration.

## Introduction

A growing body of research demonstrates that below-ground processes often increase invasive plant growth relative to native plants (Callaway and Aschehoug 2000;

Ehrenfeld and Scott 2001; van der Heijden *et al.* 2008; Parepa *et al.* 2013). This can occur through several processes. Invasives have been found to benefit from microbial symbiont accumulation (Yuan *et al.* 2014),

\* Corresponding author's e-mail address: andrew.kulmatiski@usu.edu

Published by Oxford University Press on behalf of the Annals of Botany Company.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

belowground enemy release (Kulmatiski et al. 2008), accumulation of belowground pests that decrease the growth of native competitors (Eppinga et al. 2006; Mangla et al. 2008), novel weapons (i.e. allelopathy; Bais et al. 2003) and increased nutrient cycling rates caused by disturbance or the invasive (typically non-native) plants themselves (Ehrenfeld and Scott 2001; Hawkes et al. 2005).

All of these mechanisms, with the potential exception of ‘novel weapons’, develop as a result of communication between plants and soil organisms via small organic molecules (Bais et al. 2004). Broadly, relative to native plants, invasive plants often benefit from plant–soil communication. Thus, treatments that can decrease plant–soil communication and plant–soil feedbacks can be expected to decrease invasive plant growth.

Of course, not all invasives rely on belowground processes to succeed, but for those that do, soil-based management approaches may provide novel and necessary tools for restoring native plants and the ecosystem services they provide (Kulmatiski et al. 2006; Cramer et al. 2008; Eviner and Hawkes 2008). Unfortunately, studies that determine the importance of belowground processes relative to other plant growth factors (e.g. dispersal, herbivory, etc.) are relatively rare (Kulmatiski et al. 2006). More studies are needed to determine whether or not soil-based techniques can be effective in field settings.

One recently developed belowground approach that has been used to decrease invasive plant growth is the use of activated carbon (AC). Made from coal, nutshells or wood, AC is a highly porous substance that binds organic compounds through van der Waals force. Activated carbon is essentially a cleaner form of biochar from which everything except the carbon skeleton has been removed. Activated carbon adsorbs most organic compounds, but is poor at adsorbing alcohols, glycols and non-organic compounds (e.g. ammonia and nitrate; Cheremisinoff and Ellerbusch 1978). Activated carbon is therefore different from biochar in that AC is less likely to increase microbial activity or plant growth by releasing nutrients, increasing pH or releasing volatile organics (Lehmann et al. 2011; Biederman and Harpole 2013). Activated carbon is also different from reduced organic compounds (i.e. sugar or sawdust) in that AC does not provide resources to heterotrophic organisms and is therefore not expected to affect non-native plants by slowing nutrient cycling rates (Blumenthal et al. 2003; Blumenthal 2009). Rather, the primary mechanism through which AC is likely to affect plant growth is by binding organic molecules.

Activated carbon has been used extensively in allelopathy research for its ability to bind organic molecules (Callaway and Aschehoug 2000; Inderjit and Callaway

2003; Abhilasha et al. 2008; Barney et al. 2009; Jarchow and Cook 2009; Kabouw et al. 2010). This research suggests that AC could be used to decrease the benefits of novel weapons by non-native plants. However, by indiscriminately binding organic molecules, AC may have a greater effect on plant growth by slowing communication among soil organisms (Kulmatiski and Beard 2006; Lau et al. 2008; Schertzer et al. 2009; Wurst and Van Beersum 2009; Kulmatiski 2011; Del Fabbro et al. 2014). In support of this idea, Abhilasha et al. (2008) found that AC effects were sometimes present in live soils but not in sterilized soils, indicating that AC effects were sometimes caused by plant–microbe interactions. Further, Kulmatiski (2011) found that AC addition changed soil microbial community composition and subsequent plant growth. The relative importance of these mechanisms remains unknown but in either case (i.e. allelopathy or plant–microbe communication), AC may be expected to decrease non-native growth.

In a rare field test using AC, Kulmatiski and Beard (2006) found that high concentrations (i.e.  $1000 \text{ g m}^{-2}$ ) of AC decreased non-native plant growth and increased native plant growth in small field plots. That research suggested that AC effects were caused by decreasing either allelopathy or plant–microbe communication but not likely due to changes in nutrient availability (Kulmatiski 2006, 2011). The goals of the present study were 3 folds: (i) to test AC effectiveness in restoring native plant communities in large-scale treatments, (ii) to identify the lowest effective AC concentration and (iii) to determine whether AC effects on plant growth are microbially mediated or caused by influencing the effects of allelopathy. To address the first and second goals, we monitored plant composition in large ( $15 \text{ m} \times 15 \text{ m}$ ) plots treated with different concentrations of AC and control plots in nine non-native-plant-dominated fields. We used two approaches to address the third goal. First, we assessed how AC treatments and control plots affected soil microbial composition using pyrosequencing to describe the archaeal and bacterial communities. Second, to determine whether AC effects required living microbial communities, plant growth in live and sterilized AC-treated soils was compared in a greenhouse experiment.

## Methods

### Field experiment

To test the effectiveness of AC in the field at a large scale, an experiment was conducted in nine former agricultural fields located in the Methow Valley, WA, USA ( $48^{\circ}37'N$ ,  $107^{\circ}10'W$ , 580–880 m above sea level). Fields were on Newbon-Conconully soil series association (coarse-loamy, mixed mesic Typic Haploxerolls). This semi-arid shrub-steppe

ecotype has a mean annual precipitation of 380 mm, primarily occurring as snow outside of the growing season (October–March).

The vegetation and management history of the study area is representative of a significant portion of the northern Intermountain West; abandoned agricultural fields are dominated by non-native plants (Sheley and Petroff 1998). Dominant non-native species include grasses: *Bromus inermis* Leyss. DC, *Bromus tectorum* L. and *Poa bulbosa* L.; and forbs: *Cardaria draba* L. Desv., *Centaurea diffusa* Lam., *Lactuca serriola* L., *Medicago sativa* L. and *Sisymbrium* spp. (*S. altissimum* L. and *S. loeselli* L.). Native vegetation areas that have never been used for agriculture surround these abandoned agricultural fields and include grasses: *Festuca idahoensis* Elmer., *Koeleria macrantha* (Ledeb.) Schult. and *Pseudoroegneria spicata* Pursh.; forbs: *Balsamorhiza sagittata* Pursh. and *Lupinus* spp. (*L. arbustus* Dougl., *L. aridis* Dougl. and *L. sericeus* Pursh.) and shrubs: *Artemisia tridentata* Nutt and *Purshia tridentata* Pursh. (Kulmatiski 2006). Species in this area can be categorized as non-native or native [see **Supporting Information**]. Native species are the ones commonly found in undisturbed native-dominated fields. Non-native species include non-native species and some native annual forbs, for example *Amsinckia menziesii*, which are found almost exclusively in non-native plant-dominated abandoned agricultural fields. Species that are considered non-native are actively managed by private and state landowners in the study area.

At each of the nine sites, seven 15 m × 15 m plots were established with a 5-m buffer between plots and the following treatments were applied randomly: four concentrations of coal-based AC, 100 g m<sup>-2</sup> (100), 400 g m<sup>-2</sup> (400), 700 g m<sup>-2</sup> (700), 1000 g m<sup>-2</sup> (1000), one wood-based AC at 1000 g m<sup>-2</sup> (1000w) and a control that received the same physical disturbance and seeding as the treatments but no AC, 0 g m<sup>-2</sup> (0). Each site also had one ‘complete’ control (CC) with the same physical disturbance but no AC or seed addition. Activated carbon used was a commercial grade, coal-based carbon powder with a 300 mesh size and iodine number (measure of pore content) >500 mg g<sup>-1</sup> and wood-based carbon with a 330 mesh size and iodine number >500 mg g<sup>-1</sup> (Carbon Activated Corporation, Compton, CA, USA).

Sites were treated in October 2010. Activated carbon was manually applied in powdered form using a push seed spreader and mixed into the top soil to 15 cm depth using two passes with a disc harrow pulled by a tractor. This soil mixing also removed standing vegetation. A mix of native seeds was broadcast by a hand seed spreader at a rate of 5.6 g m<sup>-2</sup>. The seed mix included by weight: 7.5 % *B. sagittata*, 12.6 % *Collomia grandiflora* Douglas ex Lindl., 17.9 % *F. idahoensis*, 17.4 %

*K. macrantha*, 9.0 % *L. sericeus*, 7.9 % *Lomatium dissectum* Nutt. and 27.7 % *P. spicata*, using local varieties when available (BFI Native Seeds, Moses Lake, WA, USA).

Vegetation responses to AC treatments were assessed using the point-intercept method. Vegetation was assessed in each of nine 1-m<sup>2</sup> subplots located in a 3 m × 3 m grid within each plot. Vegetation surveys were performed during the peak growing season (June) in 2011–2013. Additionally, germination success was assessed in three 10 cm by 10 cm in each of the nine subplots within each plot, in May 2011.

To determine the direct effect of AC on archaeal and bacterial communities, three soil cores (0–15 cm) were extracted from random locations from the 0 and 1000 plots and from undisturbed native-plant-dominated (native) plots that were adjacent to the study plots. Soil samples were placed on ice in the field and taken to a –20 °C freezer within 24 h. DNA from these soil samples was extracted using an Ultraclean-htp 96-well soil DNA kit (MoBio, Carlsbad, CA) from homogenized 0.5 g samples. DNA was further purified using the MinElute reaction cleanup kit (QIAGEN). DNA concentration and purity were determined using a Nanodrop ND-2000 spectrophotometer (NanoDrop, DE). Purified DNA extracts were stored at –80 °C. Amplification was performed with primers targeting the variable region V4–V5 of the 16S rRNA gene (515F and 907R) with 10 bp unique barcode on the forward primer (He et al. 2010). Amplicons were purified using AMPure XP beads (Agencourt, Beckman Coulter Indianapolis, IN), quantified using Quant-iT<sup>TM</sup>. PicoGreen Kit (Invitrogen) and then pooled in equimolar amounts. Unidirectional pyrosequencing was performed on a GS-Flex Titanium 454 platform according to the manufacturer’s directions (454 Life Sciences, Roche Applied Sciences, Branford, CT) at the USU Center for Integrated Biosystems (Fierer et al. 2012). The open source software package Quantitative Insights into Microbial Ecology (QIIME; Caporaso et al. 2010) was used to manage the analysis flow. Sequences were denoised, sorted by barcode and operational taxonomic units (OTUs) identified (97 % identity). Representative OTUs were assigned to a taxonomic identity using a reference database. The Python Nearest Alignment Space Termination tool (PyNAST) was used for multiple sequence alignment. Classification was performed to the lowest taxonomic level possible (Fierer et al. 2012). Some OTUs were only identifiable to kingdom while others were identifiable to genus.

In 2011, to determine the effect of AC on N cycling rates, N mineralization rates were determined in the 0, 100, 1000 and CC plots for all nine sites using the buried bag technique (Robertson et al. 1999). Briefly, in early June, 10, 4-cm-diameter core samples were collected to a depth of 15 cm in each plot. Five samples were processed immediately and five were placed individually into polyethylene

bags and reburied for 1 month. From each core sample, 10 g of soil was extracted in 100 mL of 2.0 M KCl. Inorganic N was determined from colorimetric analysis of KCl extracts using a Lachat autoanalyser (Lachat Instruments, Loveland, CO, USA). Rates of microbial mineralization were calculated using the change in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  [incubated  $(\text{NH}_4^+ + \text{NO}_3^-) - \text{initial field } (\text{NH}_4^+ + \text{NO}_3^-)$ ] and nitrification rates were calculated from the change in  $\text{NO}_3^-$  (incubated  $\text{NO}_3^- - \text{initial field } \text{NO}_3^-$ ).

### Greenhouse experiment

To isolate the mechanism through which AC changes plant growth, a greenhouse experiment was conducted in an Agricultural Research Station greenhouse facility at Utah State University, Logan, UT, USA, from August to November 2011. A total of 740 1-L polyethylene pots (7.6 cm width and 20.3 cm height; Stuewe & Sons, Model MT38, Tangent, Oregon, USA) were randomly assigned to one of three soil treatments: live soil with AC, sterile soil with AC and a control live soil. All pots were filled with a steam sterilized 6 : 1 ratio of sand and peat. The two treatments that received live soils (i.e. both AC live and control live) were inoculated with 5 % by volume field soil collected from the Methow Valley. Similar to the 1000 treatments in the field experiment, the two AC treatments received 1 % AC by mass, which was mixed throughout the pot. A coal-based, 300 mesh, commercial grade AC was used. To sterilize soils with minimal physical disruption, soils in ‘AC sterile’ treatments were inoculated with 5 % by volume gamma-irradiated (25 kGy) field soil prior to AC addition (JS8900 Batch Gamma Irradiator, Steris Isomedix, Temecula, CA, USA; [van Grunsven et al. 2007](#); [Berns et al. 2008](#)). As with any greenhouse experiments, sterilized soils were likely to be re-colonized by soil organisms during the experiment, though sterilized soils were expected to maintain smaller and less diverse microbial communities throughout the experiment ([Tanaka et al. 2003](#)).

One native and one non-native plant species were grown in each pot. For the plant species listed below, all possible native–non-native pairing combinations were replicated for each treatment. Each pairing was replicated 15 times in AC-live and control-live treatments, and 12 times in AC-sterile treatments. Species included the most common species found at the study site: three native grass species: *F. idahoensis*, *K. macrantha* and *P. spicata*, and two native forbs: *L. sericeus* and *B. sagittata*, and five common non-native species: the grass *B. tectorum* and four non-native forbs: *C. diffusa*, *L. serriola*, *Tragopogon dubius* Scop. and *S. altissimum*. *B. sagittata* grew in <10 % of the pots and was removed from primary analysis. Non-native plant growth in the pots in which *B. sagittata* never grew was used as a measure of the direct effects

of AC and sterilization on non-native plants (i.e. in the absence of competitors). Native seeds were purchased and local Methow Valley varieties were used when possible (BFI Native Seeds). Non-native seeds were collected from the Methow Valley in summer 2011.

Three successful germinants of each species were planted per pot. After 3 weeks, the tallest individual of each species was allowed to remain in each pot. Greenhouse conditions were at a mean temperature of 22 °C and sodium lamps were used to maintain 14 h of light daily. All pots were watered daily. Pots were rotated within the greenhouse weekly. After 3 months aboveground biomass and belowground biomass for each species in each pot were dried at 70 °C until constant weight, weighed and recorded. For consistency with the field experiment, we refer to native species in the greenhouse experiment as native and non-native species as non-native.

### Statistical analyses

Two sets of tests were conducted on the field data. First, to determine if results were similar to those from the previous small-scale experiment ([Kulmatiski and Beard 2006](#)), we tested the effect of the high concentration of AC on plant growth responses. Second, to determine at which concentration AC had measurable effects on plant growth, we tested the effects of multiple AC concentrations.

To test the effect of the high concentration treatment, treatment effects on the 0, 1000 and CC plots were tested on the percent cover of native species, the percent cover of non-native species and the ratio of native to non-native species (native : non-native) using a two-way randomized block design with repeated-measures and subsample analysis of variance (ANOVA). Fixed effects were treatment and year, and random effects were field and treatment within field.

To compare the effects of different AC concentrations, treatment effects in the 0, 100, 400, 700, 1000 and 1000w plots were tested on the percent cover of native species, non-native species and native : non-native ratio using a two-way randomized block design with repeated-measures and subsamples ANOVA. The fixed effect was treatment and year, and random effects were field and treatment within field. Treatment effects on germination, net N mineralization and nitrification rates were treated separately using a one-way randomized block design with repeated-measures and subsample ANOVA. For all tests, a post-hoc Tukey–Kramer method was used to adjust for Type I error and determine pairwise differences among least square means. Means from raw data are reported.

Differences in microbial community composition between 0, 1000 and native plots both early and late in the growing season were explored using principal



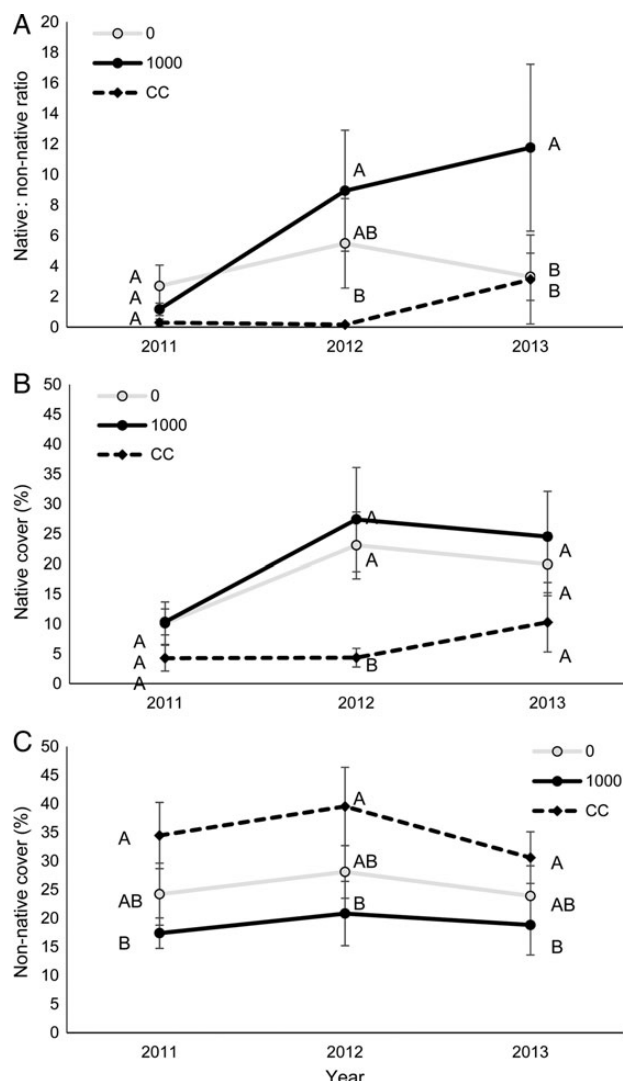
component analyses. Operational taxonomic units not present in at least 10 % of the samples were removed from analyses because such uncommon organisms were unlikely to distinguish treatments. Operational taxonomic unit abundances determined for replicate cores within a plot were averaged prior to analyses. All analyses were performed using the Vegan package in 'R' (R Core Research Team 2004). Within Vegan, a Bray–Curtis distance metric was used to calculate a distance matrix from the normalized intensity values for the OTUs to perform statistical analyses of similarities (ANOSIM; R Core Research Team 2004).

For the greenhouse experiment, effects of treatment were tested on the ratio of native:non-native above-ground biomass, native aboveground biomass, non-native aboveground biomass, native belowground biomass and non-native belowground biomass using a three-way factorial in a completely randomized design ANOVA. Fixed effects were treatment, native species and non-native species; pots were considered replicates and included in residual effects. Additionally, monoculture non-native biomass was tested in a one-way factorial with treatment and non-native species as fixed effects. Post-hoc Tukey–Kramer was used to adjust for Type I error and determine pairwise differences among least-squared means. Prior to the analysis, pots with no total growth were removed from the dataset. Above- and below-ground biomass raw values were square-root transformed and native:non-native aboveground ratio data were log-transformed to better meet assumptions of normality and homogeneity of variance. All tests were considered significant at the  $\alpha = 0.05$  level, marginally significant when  $0.05 < \alpha < 0.10$ . All significant differences indicated in figures are at the  $\alpha = 0.05$  level. All field and greenhouse analyses were conducted using the GLIMMIX procedure in SAS for Windows v. 9.3 (SAS Institute, Inc., Cary, NC, USA).

## Results

### Field experiment

**Large-scale application test.** There was an interaction effect between treatment and year for the native:non-native ratio and for native plant cover, but not for non-native plant cover ( $F_{4,696} = 7.12$ ,  $P < 0.01$ ;  $F_{4,696} = 13.49$ ,  $P < 0.01$  and  $F_{4,696} = 0.93$ ,  $P = 0.43$ , respectively; Fig. 1), so post-hoc comparisons (Tukey–Kramer adjusted) of treatment  $\times$  year combinations were examined. While there were no differences among treatments in 2011, the native:non-native ratio was greater in 1000 treatments than in CC treatments in 2012 ( $T_{1,696} = 3.31$ ,  $P = 0.03$ ) and was greater than both



**Figure 1.** Treatments CC, 0 and 1000 g m<sup>-2</sup> for each year for (A) native:non-native ratio, (B) native species percent cover and (C) non-native percent cover (mean  $\pm$  SE). Letters denote differences among treatments within years at the  $\alpha < 0.05$  level.

0 and CC treatments in 2013 ( $T_{1,696} = 3.19$ ,  $P = 0.04$  and  $T_{1,696} = 3.26$ ,  $P = 0.03$ , respectively; Fig. 1A). While there was no difference among treatments in 2011, native plant cover was greater in 1000 and 0 treatments than in CC treatments in 2012 ( $T_{1,696} = 4.82$ ,  $P < 0.01$  and  $T_{1,696} = 3.92$ ,  $P < 0.01$ , respectively; Fig. 1B). In 2013, the difference in native plant cover between 1000 and CC plots remained marginally significant ( $T_{1,696} = 2.98$ ,  $P = 0.07$ ), but native plant cover was no longer greater in 0 than CC plots ( $T_{1,696} = 2.02$ ,  $P = 0.53$ ). There was no treatment  $\times$  year interaction for non-native cover ( $F_{4,696} = 0.93$ ,  $P = 0.45$ ; Fig. 1C); non-native plant cover was greater in CC than 1000 treatments across years ( $F_{2,16} = 4.58$ ,  $P = 0.03$ ).

**Comparing different concentrations of AC.** In May 2011, there was a treatment effect on the native : non-native germination ratio ( $F_{5,40} = 3.01$ ,  $P = 0.02$ ); 1000w had a greater ratio than 100 or 700 ( $T_{1,40} = 3.02$ ,  $P = 0.05$  and  $T_{1,40} = 3.16$ ,  $P = 0.03$ ; Fig. 2A–D). 1000w also had a marginally greater native : non-native ratio than 0 and 400 treatments ( $T_{1,40} = 2.81$ ,  $P = 0.08$  and  $T_{1,40} = 2.80$ ,  $P = 0.08$ ). There was no effect of AC treatment on native species germination ( $F_{5,40} = 0.53$ ,  $P = 0.75$ ; Fig. 2E–H), but there was an effect of treatment on non-native species germination ( $F_{5,40} = 3.00$ ,  $P = 0.02$ ); 100 had greater germination than 1000w ( $T_{1,40} = 3.32$ ,  $P = 0.02$ ) and 700 was marginally greater than 1000w ( $T_{1,40} = 2.79$ ,  $P = 0.08$ ; Fig. 2I–L).

There was an interaction effect between treatment and year for peak growing season native: non-native ratio, native plant cover and non-native plant cover ( $F_{10,1245} = 6.98$ ,  $P < 0.01$ ;  $F_{10,1288} = 3.52$ ,  $P < 0.01$  and  $F_{10,1309} = 10.64$ ,  $P < 0.01$ , respectively) so post-hoc comparisons (Tukey–Kramer adjusted) of treatment  $\times$  year combinations were examined.

In June 2011, the native : non-native ratio was greater in 400 and 1000w treatments than in 700 treatments ( $T_{1,1223} = 3.51$ ,  $P = 0.05$  and  $T_{2,1223} = 3.93$ ,  $P = 0.01$ ; Fig. 2A) or 1000 treatments ( $T_{1,1223} = 3.51$ ,  $P = 0.05$  and  $T_{1,1223} = 3.93$ ,  $P = 0.01$ ). Neither native nor non-native cover differed among treatments in June 2011. In June 2012, there were no differences in the native : non-native ratio, native cover or non-native cover among treatments. In June 2013, the native : non-native ratio was greater in 400, 700 and 1000 treatments than in 0 treatments ( $T_{1,1223} = 3.64$ ,  $P = 0.03$ ;  $T_{1,1223} = 4.44$ ,  $P < 0.01$  and  $T_{1,1223} = 4.12$ ,  $P < 0.01$ , respectively) and 100 treatments ( $T_{1,1254} = 4.48$ ,  $P < 0.01$ ;  $T_{1,1254} = 4.08$ ,  $P = 0.01$  and  $T_{1,1254} = 4.61$ ,  $P < 0.01$ , respectively). Native cover did not differ among treatments in 2013. Non-native cover was greater in 0 treatments than 100 and 1000 treatments ( $T_{1,1288} = 3.68$ ,  $P = 0.03$  and  $T_{1,1288} = 3.59$ ,  $P = 0.04$ , respectively).

#### Soil N and microbial responses to field treatments.

There was no treatment effect for net N mineralization or nitrification rates ( $F_{3,21} = 0.37$ ,  $P = 0.78$  and  $F_{3,21} = 0.67$ ,  $P = 0.58$ ). Mean final N levels were  $0.35 \text{ mg NH}_4^+ \text{ kg}^{-1} \text{ soil}$  and  $1.23 \text{ mg NO}_3^- \text{ kg}^{-1} \text{ soil}$ , with a mean net N mineralization rate of  $0.029 \text{ mg kg}^{-1} \text{ day}^{-1}$  and a mean nitrification rate of  $0.028 \text{ mg kg}^{-1} \text{ day}^{-1}$ .

Between early and late sampling, microbial community composition in all treatments became more negative along PC axis 1 (Fig. 3; ANOSIM statistic = 0.21,  $P = 0.01$ ). Early in the season, AC made microbial communities similar to native soil. This was due to decreases in an unidentified bacteria and an Actinomycetales, and increases in

aMicrococcaceae, a Pseudomonas and a Flavobacterium species (Fig. 2, ANOSIM statistic = 0.21,  $P = 0.01$ ). Late in the season, native soils were distinguished from 0 and 1000 soils by an increase in the unidentified bacteria and Actinomycetales and a decrease in Flavobacterium.

#### Greenhouse experiment

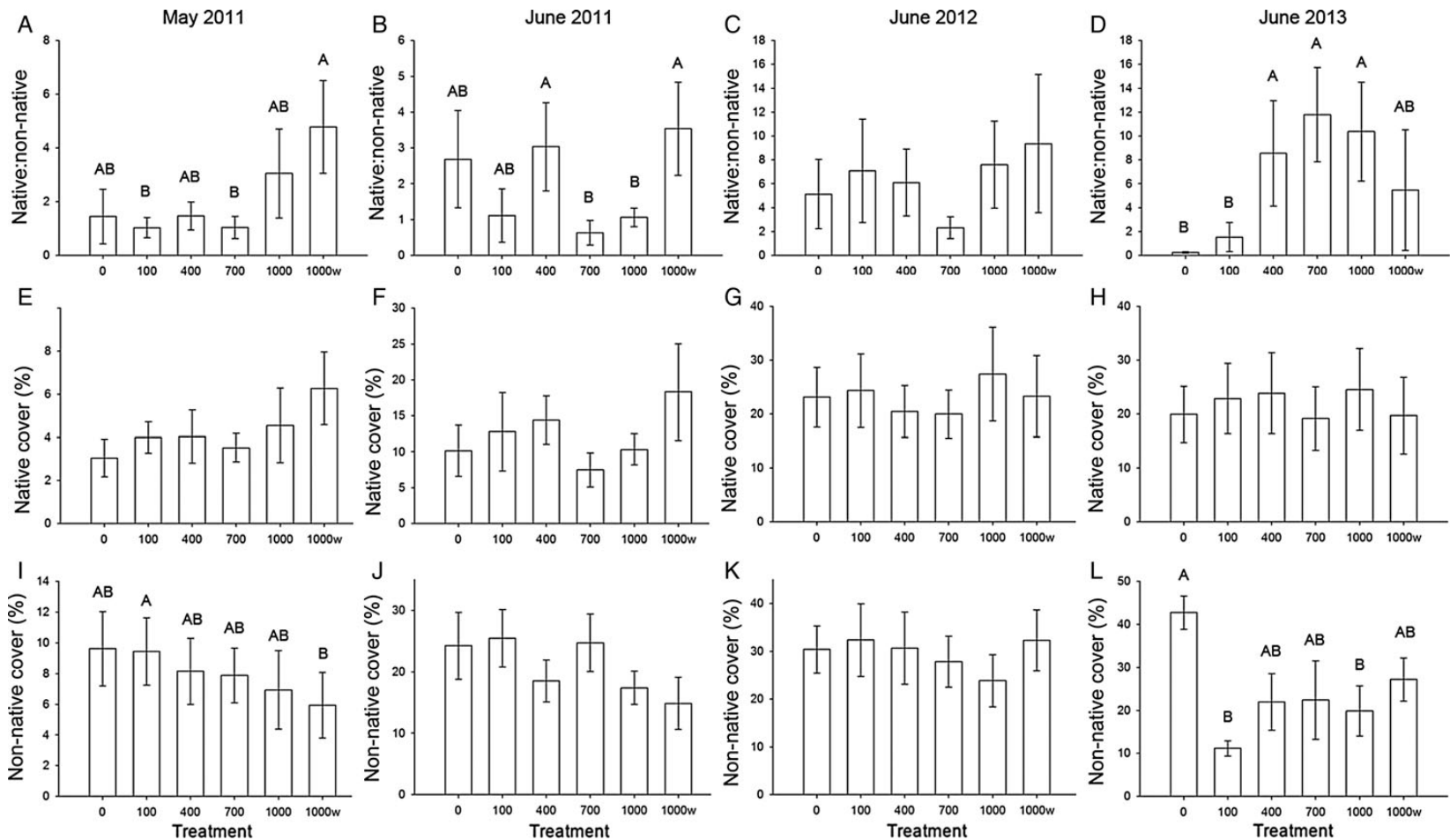
The native : non-native aboveground biomass ratio was greater in AC live soil than AC sterile and control soils ( $F_{2,657} = 3.29$ ,  $P = 0.04$ ; Fig. 4A). Native aboveground biomass in AC live soil was lower than native aboveground biomass in the control soil, while native aboveground biomass did not differ between AC sterile and AC live or control soil ( $F_{2,657} = 5.41$ ,  $P < 0.01$ ; Fig. 4B). Non-native aboveground biomass was lower in AC live soil than in AC sterile and control soils ( $F_{2,657} = 6.06$ ,  $P < 0.01$ ; Fig. 4B). This was also true for non-native species grown in monoculture (i.e. unsuccessful *B. sagittata* pairings;  $F_{2,182} = 5.18$ ,  $P < 0.01$ ; Fig. 5).

Native species growth differed among non-native pairings ( $F_{4,657} = 10.95$ ,  $P < 0.01$ ). Natives had the greatest biomass with *S. altissimum* and the least with *B. tectorum*. Non-native species growth similarly differed among native pairings ( $F_{3,657} = 4.48$ ,  $P < 0.01$ ). Non-natives had the greatest aboveground biomass with *L. sericeus* and the least with *K. macrantha*.

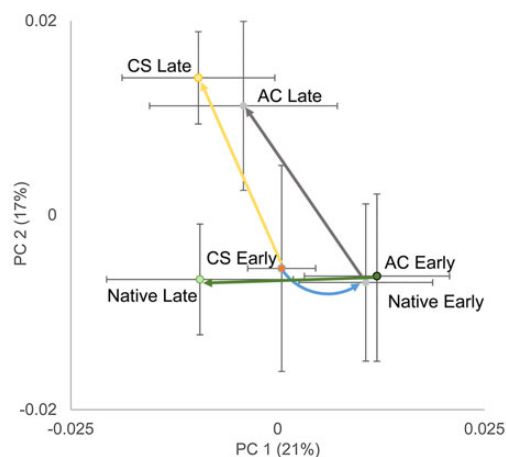
Belowground native biomass and non-native biomass showed no response to treatment ( $F_{2,657} = 1.83$ ,  $P = 0.16$  and  $F_{2,657} = 1.51$ ,  $P = 0.22$ , respectively).

#### Discussion

In both field and greenhouse experiments, AC decreased non-native plant growth and increased the relative abundance of native to non-native plants. At the end of the 3-year field experiment, this effect was evident at AC concentrations  $>400 \text{ g m}^{-2}$  (Fig. 2D). In the greenhouse experiment, the fact that AC decreased non-native growth in monoculture pots (Fig. 5) indicated that AC effects occurred regardless of competition or allelopathy. The fact that non-native growth decreased only in live soils suggested that AC effects were microbially mediated (Figs 4 and 5). The fact that AC decreased non-native growth suggested that AC either increased negative plant–microbe interactions or decreased positive plant–microbe interactions. Because AC is known to indiscriminately bind the organic molecules that allow communication and the development of interactions among soil organisms, we suggest that the most likely explanation for the microbially mediated decrease in non-native plant growth in AC plots is that AC decreased the development of positive plant–microbe interactions that have recently been suggested to dominate plant–soil interactions



**Figure 2.** (A–L) Treatments 0, 400, 700, 1000 and 1000w g AC m<sup>-2</sup> for May 2011, June 2011, June 2012 and June 2013 for native : non-native species cover ratio, native species percent cover and non-native species percent cover (mean ± SE). Letters denote significant differences between treatments at the  $\alpha < 0.05$  level.



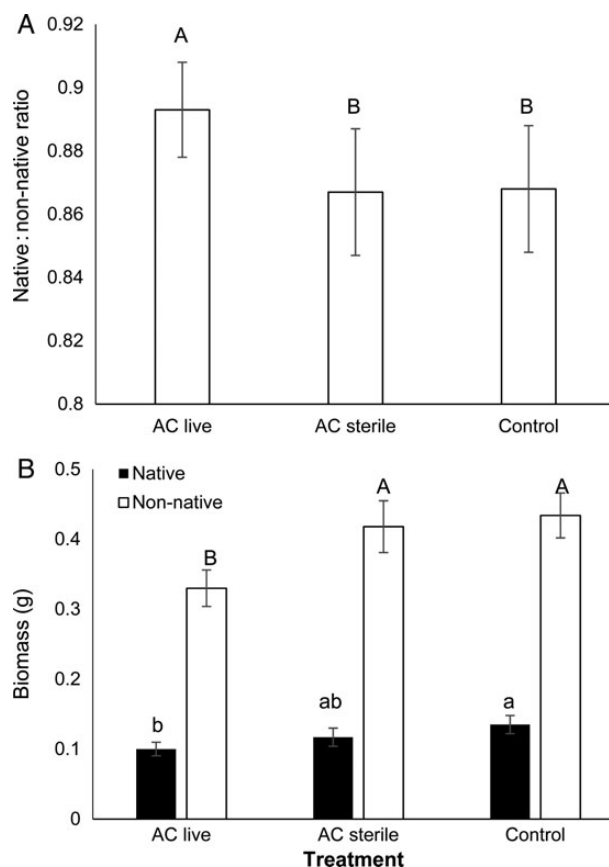
Organism	PC 1 Weight	Organism	PC 2 Weight
K: Bacteria	0.74	F: Gaiellaceae	0.65
O: Actinomycetales	0.29	G: Flavobacterium	-0.38
F: Micrococcaceae	-0.23	K: Bacteria	-0.27
G: Pseudomonas	-0.20	O: Solirubrobacterales	0.25
G: Flavobacterium	-0.18	O: Actinomycetales	-0.21

**Figure 3.** Principal component analysis of archaeal and bacterial OTUs associated with native plant communities (native), control + seed-treated soils (CS) and activated-carbon-treated (AC) soils both early in the growing season prior to plant growth (early) and near the end of the growing season (late). The blue arrow shows the effect of adding AC to treated soils. The grey, yellow and green arrows show how microbial communities changed over the growing season in AC-treated, seeded and native plots, respectively. The table inset shows the bacteria associated with PC1 and PC2. K = kingdom, F = family, O = order and G = genus.

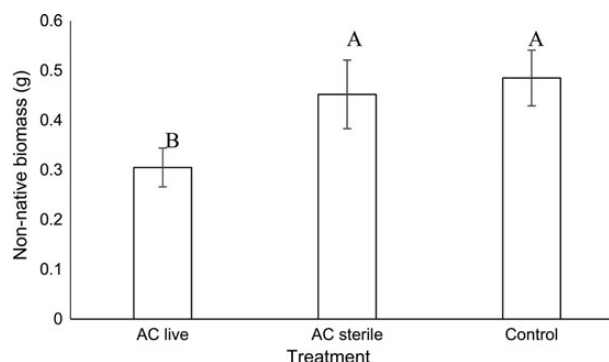
(Kulmatiski et al. 2014). Microbial analyses suggested organisms that may play a role in these plant–microbe interactions.

Similar to previous research conducted on a smaller spatial scale (1 m × 1 m plots), we found that the addition of 1000 g m<sup>-2</sup> of coal-based AC to larger plots (15 m × 15 m) using more real-world application techniques (i.e. tractor discing rather than hand mixing) increased the ratio of native to non-native plant species (Kulmatiski and Beard 2006; Kulmatiski 2011). Non-native species were the drivers of this community composition change (Figs 1 and 2). More specifically, non-native cover decreased from 24 % cover in seeded controls to 19 % cover in plots treated with 1000 g m<sup>-2</sup> coal-based AC. This effect of AC was similar to the effect of adding native seed: adding native seed decreased non-native cover from 31 to 24 %.

Greenhouse results provided insight into the potential mechanisms through which AC may increase the relative abundance of native plant growth. A number of positive and negative interactions occur among plant roots, soils



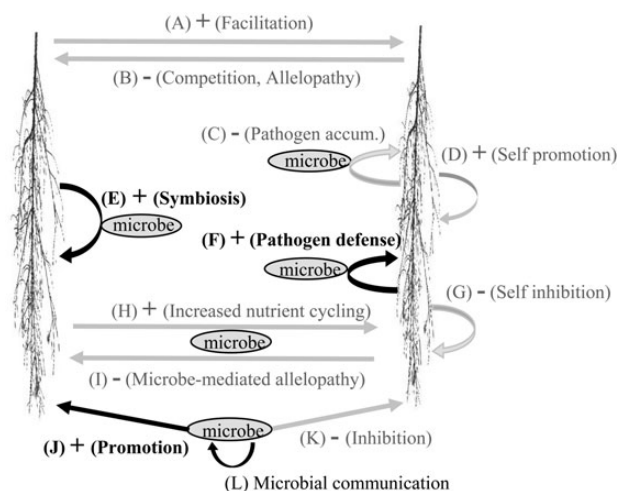
**Figure 4.** (A) Native : non-native species aboveground biomass ratio in control, activated-carbon-treated (AC live) and activated-carbon-treated and sterilized (AC sterile) soils. (B) Total aboveground biomass for all native species and non-native species in control, activated-carbon-treated (AC live) and activated-carbon-treated and sterilized (AC sterile) soils (mean ± SE). Letters denote differences among treatments at the  $\alpha < 0.05$  level.



**Figure 5.** Non-native biomass (g) for individuals grown in monoculture in control, activated-carbon-treated (AC live) and activated-carbon-treated and sterilized (AC sterile) soils (mean ± SE). Letters denote differences among treatments at the  $\alpha < 0.05$  level.

and soil organisms that can affect plant growth (Fig. 6). Because AC binds organic molecules we suggest that AC is likely to decrease plant–soil communication and subsequent interactions. Therefore, we suggest that the





**Figure 6.** Potential plant–soil interactions that may be affected by activated carbon soil additions. Pathways supported by this research are shown in black. Pathways not supported by this research are shown in grey.

decrease in plant growth caused by AC was more likely to be caused by decreases in positive plant–soil interactions (i.e. E, F and J in Fig. 6) than by increases in negative plant–soil interactions (i.e. B, C, G, I and K in Fig. 6). Results from our sterilization experiment in the greenhouse indicated that AC effects were only realized in live soils. Thus, AC did not appear to affect plant growth by suppressing facilitation or self-promotion (i.e. A and D in Fig. 6). The most likely potential mechanisms remaining are suppression of symbioses, suppression of pathogen defence and suppression of increased nutrient cycling (Hawkes et al. 2005). Results from our current and previous field experiments suggest that AC does not increase nutrient availability (Kulmatiski and Beard 2006). Therefore, we suggest that the most likely mechanisms through which AC affects plant growth is by suppressing (i) the development of symbiotic relationships between plants and soil organisms (Callaway and Aschehoug 2000; Mangla et al. 2008; Weißhuhn and Prati 2009; Wurst and Van Beersum 2009; Kulmatiski 2011) or (ii) the pathogen defence (Van der Putten 2003; Bais et al. 2004; Oriens and Ward 2010; Doornbos et al. 2012). Future experiments would be needed to distinguish the importance of these two mechanisms.

Many experiments have been conducted testing allelopathic effects using AC (Lau et al. 2008; Wurst et al. 2010; Murrell et al. 2011); however, the results of our experiments suggest that for many of the dominant native and non-native species of the western USA, the effects of AC on plant–microbial interactions are more influential than its effects on allelopathy.

Results from microbial analyses are consistent with the idea that AC affects plant growth by changing microbial

composition. Early in the spring, before plant growth, microbial communities in AC-treated soils ( $1000 \text{ g AC m}^{-2}$ ) were more similar to undisturbed native soils than to control soils ( $0 \text{ g AC m}^{-2}$ ). More specifically, early season AC soils and native soils were associated with an increase in an unidentified bacterium and Actinomycetales, and with a decrease in a Flavobacterium. However, late season, only native soils were associated with these OTUs. This suggested that AC created a soil microbial community that was similar to native plants, but that once non-native plants grow on these soils the microbial community returns to a composition more similar to that associated with non-native plants. Flavobacterium have been noted for their plant growth promoting activity (Soltani et al. 2010), so a decrease in Flavobacterium is consistent with a decrease in positive plant–microbe interactions. Actinomycetales have been found to affect fungal infection (Maier et al. 2004), so it is possible that AC treatments increased Actinomycetales, which either increased fungal pathogen infection or decreased mycorrhizal infection. These potential mechanisms are purely speculative, but help narrow down a long list of soil organisms to those that are more likely to have important effects on plant growth. Follow-up experiments testing the effects of these different organisms (i.e. tests of Koch's postulates) will be needed to test their effects on native and non-native plant growth.

Two other goals of this experiment were to test the effectiveness of (i) lower concentrations of AC and (ii) wood-based AC. We wanted to test the effectiveness of wood-based carbon because wood-based AC treatments would hold the benefit of fixing carbon into soils and may allow the development of on-site production. On-site carbon production could have the benefit of both removing woody materials that present a fire hazard while simultaneously fixing carbon in the soil. In both cases results were variable and therefore difficult to interpret. In the third year of the study, the native: non-native ratios were greater in 400, 700 and 1000 treatments suggesting that at least  $400 \text{ g m}^{-2}$  of AC are needed for native plant restoration. Results, however, were not consistent across years so further testing with different concentrations in different sites is recommended. Plant responses to wood-based AC were even more variable, with wood-based AC having a greater native: non-native ratio than coal-based AC in the first year and a lower native: non-native ratio than coal-based AC in the third year. It is difficult, therefore, to conclude whether or not wood-based AC is a suitable alternative to coal-based AC.

Native plant responses were not as strong in this study as in the previous smaller-scale field experiment (Kulmatiski and Beard 2006). This may have been because the larger-scale application techniques (i.e. tractor discing

rather than hand mixing) used in the current study were likely to produce less consistent AC incorporation into the soil. Additionally, more than twice as much rain fell during the first spring of the large-scale experiment relative to the small-scale experiment (i.e. 21.5 versus 9.3 cm precipitation between March and May; Kulmatiski 2011). As a result, native plants established vigorously in control plots during the large-scale study, making it difficult for any treatments to increase native plant growth beyond seeding success. The fact that AC effects were observed in the greenhouse, small-scale study (Kulmatiski and Beard 2006) and the current large-scale study suggests that AC effects are consistent across a wide range of conditions.

There is rapidly growing interest in the use of biochar (Atkinson et al. 2010; Lehmann et al. 2011; Spokas et al. 2012). Biochar has typically been used to increase plant growth in agricultural settings. The positive effect of biochar has been attributed to the fact that biochar can increase N, release P, increase pH and release ethylene, among other mechanisms (Spokas et al. 2010; Lehmann et al. 2011; Biederman and Harpole 2013). Consistent with this ‘fertilization’ effect, biochar increases annual plant growth more than perennial plant growth (Biederman and Harpole 2013). Our results suggest that AC effects on plant growth are fundamentally different than biochar effects on plant growth. Our results suggest that AC decreases plant growth by suppressing beneficial plant–microbe interactions, and that this benefits native plants by suppressing the growth of weeds that rely on fast growth rates. Thus, we suggest that most biochar is unlikely to be weed suppressive unless it has a very high C : N ratio (e.g. > 300 : 1) or it is applied at very high rates (i.e. > 5 % by volume of soil) that can interfere with plant–microbe communication (Biederman and Harpole 2013).

It is necessary to consider the cost of AC application before considering broad use. Costs for tested AC concentrations are approximately: 100 g m<sup>-2</sup>: \$454 ha<sup>-1</sup>, 400 g m<sup>-2</sup>: \$1817 ha<sup>-1</sup>, 700 g m<sup>-2</sup>: \$3180 ha<sup>-1</sup>, 1000 g m<sup>-2</sup>: \$4543 ha<sup>-1</sup> and 1000 w g m<sup>-2</sup>: \$5618 ha<sup>-1</sup>. Due to its high cost, AC is not a practical large-scale restoration tool. However, it may be useful for targeted treatments, in relatively small sites, such as restoring abandoned oil pad sites. Because AC is eliciting community changes by decreasing positive microbial interactions with non-native species, it is likely more beneficial to further research and explore techniques that manipulate particular plant–microbe interactions (i.e. through inoculation).

## Conclusions

In a large-scale field application, AC increased native restoration even when applied in a year with a wet spring that resulted in vigorous native plant growth. Previous

results from a small-scale experiment suggested that this effect may be greater in drier years. A greenhouse experiment suggested that AC decreases non-native plant growth by suppressing the development of positive plant–microbe interactions—not by suppressing allelopathy or nutrient cycling rates. Bacterial analyses suggested that Actinomycetales and Flavobacterium are good candidates for future testing of AC effects. Activated carbon application remains a blunt and costly restoration tool that may only have applications in high-value lands, but results demonstrate the potential for soil-based manipulations that can be used to guide plant community development.

## Sources of Funding

The work was funded by the USDA NRI (# 2010-85320-20402) and the USU Ecology Center. This research was supported by the Utah Agricultural Experiment Station, Utah State University and was approved as journal paper number 8701.

## Contributions by the Authors

N.E.N. analysed the data and led the writing. A.K. and K.H.B. conceived the experiments and oversaw the collection of field and lab data. J.M.N. oversaw the microbial component. A.K. was supported by a USDA AFRI grant.

## Conflicts of Interest Statement

None declared

## Acknowledgements

The authors thank the Washington Department of Fish and Game for use of land and resources for the field experiment; the USDA Agricultural Research Station for greenhouse space; Brianne Loya, Andrew McNowen, Anna Peschel and Maureen Schweer for assistance with field and lab work; and Peter Adler for comments on the manuscript.

## Supporting Information

The following supporting information is available in the online version of this article –

**Table S1.** Species grouping and classification for field experiment results.

## Literature Cited

Abhilasha D, Quintana N, Vivanco J, Joshi J. 2008. Do allelopathic compounds in invasive *Solidago canadensis* s.l. restrain the native European flora? *Journal of Ecology* **96**:993–1001.

- Atkinson C, Fitzgerald J, Hipps N. 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and Soil* **337**:1–18.
- Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* **301**:1377–1380.
- Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM. 2004. How plants communicate using the underground information superhighway. *Trends in Plant Science* **9**:26–32.
- Barney JN, Sparks JP, Greenberg J, Whitlow TH, Guenther A. 2009. Biogenic volatile organic compounds from an invasive species: impacts on plant–plant interactions. *Plant Ecology* **203**:195–205.
- Berns AE, Philipp H, Narres HD, Burauel P, Vereecken H, Tappe W. 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. *European Journal of Soil Science* **59**:540–550.
- Biederman LA, Harpole WS. 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *Global Change Biology Bioenergy* **5**:202–214.
- Blumenthal DM. 2009. Carbon addition interacts with water availability to reduce invasive forb establishment in a semi-arid grassland. *Biological Invasions* **11**:1281–1290.
- Blumenthal DM, Jordan NR, Russelle MP. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. *Ecological Applications* **13**:605–615.
- Callaway RM, Aschehoug ET. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* **290**:2075.
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**:335–336.
- Cheremisinoff PN, Ellerbusch F. 1978. *Carbon absorption handbook*. Ann Arbor, MI: Ann Arbor Science Publishers.
- Cramer VA, Hobbs RJ, Standish RJ. 2008. What's new about old fields? Land abandonment and ecosystem assembly. *Trends in Ecology and Evolution* **23**:104–112.
- Del Fabbro C, Guesewell S, Prati D. 2014. Allelopathic effects of three plant invaders on germination of native species: a field study. *Biological Invasions* **16**:1035–1042.
- Doornbos RF, van Loon LC, Bakker PA. 2012. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agronomy for Sustainable Development* **32**:227–243.
- Ehrenfeld JG, Scott N. 2001. Invasive species and the soil: effects on organisms and ecosystem processes. *Ecological Applications* **11**:1259–1260.
- Eppinga MB, Rietkerk M, Dekker SC, De Ruiter PC, Van der Putten VH. 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. *Oikos* **114**:168–176.
- Eviner VT, Hawkes CV. 2008. Embracing variability in the application of plant–soil interactions to the restoration of communities and ecosystems. *Restoration Ecology* **16**:713–729.
- Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R. 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME Journal* **6**:1007–1017.
- Hawkes CV, Wren IF, Herman DJ, Firestone MK. 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters* **8**:976–985.
- He ZL, Xu MY, Deng Y, Kang SH, Kellogg L, Wu LY, Van Nostrand JD, Hobbie SE, Reich PB, Zhou JZ. 2010. Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO<sub>2</sub>. *Ecology Letters* **13**:564–575.
- Inderjit, Callaway RM. 2003. Experimental designs for the study of allelopathy. *Plant and Soil* **256**:1–11.
- Jarchow ME, Cook BJ. 2009. Allelopathy as a mechanism for the invasion of *Typha angustifolia*. *Plant Ecology* **204**:113–124.
- Kabouw P, Nab M, Van Dam NM. 2010. Activated carbon addition affects substrate pH and germination of six plant species. *Soil Biology and Biochemistry* **42**:1165–1167.
- Kulmatiski A. 2006. Exotic plants establish persistent communities. *Plant Ecology* **187**:261–275.
- Kulmatiski A. 2011. Changing soils to manage plant communities: activated carbon as a restoration tool in ex-arable fields. *Restoration Ecology* **19**:102–110.
- Kulmatiski A, Beard KH. 2006. Activated carbon as a restoration tool: potential for control of invasive plants in abandoned agricultural fields. *Restoration Ecology* **14**:251–257.
- Kulmatiski A, Beard KH, Stark JM. 2006. Soil history as a primary control on plant invasion in abandoned agricultural fields. *Journal of Applied Ecology* **43**:868–876.
- Kulmatiski A, Beard KH, Stevens J, Cobbold S. 2008. Plant–soil feedbacks: a meta-analytical review. *Ecology Letters* **11**:980–992.
- Kulmatiski A, Anderson-Smith A, Beard KH, Doucette-Riise S, Mazzacavallo M, Nolan NE, Ramirez RA, Stevens JR. 2014. Most soil trophic guilds increase plant growth: a meta-analytical review. *Oikos* **123**:1409–1419.
- Lau JA, Puliafico KP, Kopshever JA, Steltzer H, Jarvis EP, Schwarzlender M, Strauss SY, Hufbauer RA. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytologist* **178**:412–423.
- Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D. 2011. Biochar effects on soil biota—a review. *Soil Biology and Biochemistry* **43**:1812–1836.
- Maier A, Riedlinger J, Fiedler H-P, Hampp R. 2004. Actinomycetales bacteria from a spruce stand: characterization and effects on growth of root symbiotic and plant parasitic soil fungi in dual culture. *Mycological Progress* **3**:129–136.
- Mangla S, Inderjit, Callaway RM. 2008. Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *Journal of Ecology* **96**:58–67.
- Murrell C, Gerber E, Krebs C, Parepa M, Schaffner U, Bossdorf O. 2011. Invasive knotweed affects native plants through allelopathy. *American Journal of Botany* **98**:38–43.
- Orians CM, Ward D. 2010. Evolution of plant defenses in nonindigenous environments. *Annual Review of Entomology* **55**:439–459.
- Parepa M, Schaffner U, Bossdorf O. 2013. Help from under ground: soil biota facilitate knotweed invasion. *Ecosphere* **4**:31. <http://dx.doi.org/10.1890/ES13-00011.1>.
- R Core Research Team. 2004. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

- Robertson GP, Coleman DC, Bledsoe CS, Sollins P (eds). 1999. *Standard soil methods for long-term ecological research*. New York: Oxford University Press.
- Schertzer JW, Boulette ML, Whiteley M. 2009. More than a signal: non-signaling properties of quorum sensing molecules. *Trends in Microbiology* **17**:189–195.
- Sheley RL, Petroff JK. 1998. *Biology and management of noxious rangeland weeds*. Corvallis: Oregon State University Press.
- Soltani A-A, Khavazi K, Asadi-Rahmani H, Omidvari M, Abaszadeh Dahaji P, Mirhoseyni H. 2010. Plant growth promoting characteristics in some *Flavobacterium* spp. isolated from soils of Iran. *Journal of Agricultural Science* **2**:106–110.
- Spokas KA, Baker J, Reicosky D. 2010. Ethylene: potential key for biochar amendment impacts. *Plant and Soil* **333**:443–452.
- Spokas KA, Cantrell KB, Novak JM, Archer DW, Ippolito JA, Collins HP, Boateng AA, Lima IM, Lamb MC, McAloon AJ, Lentz RD, Nichols KA. 2012. Biochar: a synthesis of its agronomic impact beyond carbon sequestration. *Journal of Environmental Quality* **41**:973–989.
- Tanaka S, Kobayashi T, Iwasaki K, Yamane S, Maeda K, Sakurai K. 2003. Properties and metabolic diversity of microbial communities in soils treated with steam sterilization compared with methyl bromide and chloropicrin fumigations. *Soil Science and Plant Nutrition* **49**:603–610.
- van der Heijden MGA, Bardgett RD, van Straalen NM. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**:296–310.
- Van Der Putten WH. 2003. Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology* **84**:2269–2280.
- van Grunsven RHA, van der Putten WH, Bezemer TM, Tamis WLM, Berendse F, Veenendaal EM. 2007. Reduced plant–soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology* **95**:1050–1057.
- Weißhuhn K, Prati D. 2009. Activated carbon may have undesired side effects for testing allelopathy in invasive plants. *Basic and Applied Ecology* **10**:500–507.
- Wurst S, Van Beersum S. 2009. The impact of soil organism composition and activated carbon on grass–legume competition. *Plant and Soil* **314**:1–9.
- Wurst S, Vender V, Rillig MC. 2010. Testing for allelopathic effects in plant competition: does activated carbon disrupt plant symbioses? *Plant Ecology* **211**:19–26.
- Yuan Y, Tang J, Leng D, Hu S, Yong JWH, Chen X. 2014. An invasive plant promotes its arbuscular mycorrhizal symbioses and competitiveness through its secondary metabolites: indirect evidence from activated carbon. *PLoS ONE* **9**:e97163.