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Research Paper

Evaluation of different carbon sources for anaerobic soil disinfestation against *Rhizoctonia solani* on lettuce in controlled production systems

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Summary. Effects of anaerobic soil disinfestation (ASD) on *Rhizoctonia solani* basal rot of lettuce were assessed considering: two soil types; different C-sources; different temperature regimes; two treatment durations; and two lettuce crop cycles, in the presence of a high disease incidence from artificial infestation with the pathogen. C-source, temperature, and incubation period, and their interaction, affected the efficacy of the ASD treatment for the lettuce-*R. solani* pathosystem, with differences depending on the soil type. *Brassica carinata* pellets, used as a C-source, reduced incidence of *Rhizoctonia* basal rot in the first crop cycle by 50 to 69% in a peat soil after 3 weeks of treatment at 21°C, and by 52 to 60% after 3 weeks of treatment at 26 or 31°C, compared to the inoculated and untreated experimental controls without anaerobic conditions. The best disease reduction was provided by *B. carinata* pellets applied, under anaerobic conditions, to peat soil (79% efficacy) and a sandy loam soil (100% efficacy) kept at 31°C for 6 weeks. Generally, ASD based on *B. carinata* pellets provided greater disease reduction in the first crop cycle than the second, with the only exception being for results achieved in both soils incubated for 6 weeks at 21, 26 and 31°C. Wild rocket used as a C-source provided the greatest disease reduction (78–83%) on plants grown in peat soil at the first crop cycle after 6 weeks of the ASD treatment at 31°C, while wild rocket provided disease reduction of 29 and 50% when mixed with the sandy-loam soil under the same conditions for 6 weeks. The efficacy of the ASD treatment with compost was improved in the second crop cycle, compared to the first, resulting in the greatest disease reduction (52 and 66% efficacy) reached in the sandy-loam soil incubated for 3 weeks at 21°C, and 63% efficacy in peat soil previously treated for 6 weeks at 26 and 31°C. However, slight reductions in lettuce plant development was generally evident in the first crop cycle, which could be due to differences in efficacy of the tested ASD treatments and to phytotoxicity. The greatest yield from sandy loam soil was obtained for the *B. carinata* pellets and wild rocket at 26 and 31°C in the second cultivation cycle. Partial efficacy of ASD does not justify the adoption of this method against *R. solani* on lettuce under intensive crop systems. However, ASD based on *Brassicaceae* and compost as carbon source applied in a sandy-loam soil may be valuable for reducing *R. solani* incidence, at lower temperatures than those required for soil solarisation or biosolarisation.

Keywords. *Lactuca sativa*, pre-plant treatments, soil-borne pathogens, biological soil disinfestation.

INTRODUCTION

Anaerobic soil disinfestation (ASD) is a method based on the anaerobic decomposition of organic matter, and this was developed as a possible alternative to the use of fumigants (Shennan *et al.*, 2014). ASD is based on two systems, one developed in the Netherlands, as a biological soil disinfestation (Blok *et al.*, 2000), and the other in Japan, as a soil reductive sterilisation process (Shinmura, 2004). This is applied in Japan, the Netherlands, the United States of America, and Argentina, to control of different pathogens and pests under practical conditions (Roskopf *et al.*, 2015; Shrestha *et al.*, 2016; Shennan *et al.*, 2018).

The ASD process is based on the addition of labile carbon sources to the soil to stimulate microbial growth and respiration, followed by irrigation to fill the water pore spaces, and covering the soil with plastic films, to reduce gas exchange. This then permits diffusion through the soil of by-products of decomposition, as well as reduced soil oxygen (Butler *et al.*, 2012a, 2012b; Shennan *et al.*, 2014). These treatments establish anaerobic conditions, as the aerobic microorganisms consume the remaining oxygen present in the soil, and the microbial communities shift to facultative and obligate anaerobes (Mazzola *et al.*, 2007; Momma *et al.*, 2010; Mowlick *et al.*, 2012, 2013; Huang *et al.*, 2016; Hewavitharana and Mazzola, 2016). After the anaerobic conditions have been maintained for a period, depending on soil temperature and the type of C-source used, the soil is uncovered to allow oxygen to return, stimulating the degradation of the remaining anaerobic decomposition by products (Shennan *et al.*, 2014). ASD is effective against soil-borne pathogens and pests through different mechanisms, including production of organic acids via anaerobic decomposition of the added C, production of volatiles (Okazaki and Nose, 1986; Momma *et al.*, 2006; Mazzola and Hewavitharana, 2014; Huang *et al.*, 2016), and biocontrol activity of fungal and bacterial communities that grow during the process (Momma *et al.*, 2010; Mazzola and Manici, 2012; Mowlick *et al.*, 2012, 2013; Runia *et al.*, 2012, 2014; Butler *et al.*, 2014 a, 2014b).

Most studies and practical applications of ASD have dealt with crops such as asparagus, tomato, pepper, eggplant, cucumber, melon, spinach, strawberry, cut flowers and fruit trees (Blok *et al.*, 2000; Mazzola *et al.*, 2001; Goud *et al.*, 2004; Messiha *et al.*, 2007; Yossen *et al.*, 2008; Lamers *et al.*, 2010; Mazzola and Manici, 2012; Butler *et al.*, 2012 a, 2012b; Mowlick *et al.*, 2013; Hewavitharana and Mazzola, 2016; Serrano-Pérez *et al.*, 2017; Shennan *et al.*, 2014, 2018). The

C-sources used generally depends on the availability of inexpensive or waste materials in the different locations, and there is variability among countries. Among the tested C-sources, the most common have been: wheat bran (Yossen *et al.*, 2008; Momma *et al.*, 2010), rice bran (Shennan *et al.*, 2010; Strauss and Kluepfel, 2015), ryegrass (Blok *et al.*, 2000; Goud *et al.*, 2004), molasses (Momma *et al.*, 2010, 2013; Butler *et al.*, 2012 a), diluted ethanol (Momma *et al.*, 2010; Hewavitharana and Mazzola, 2013), green manure (Butler *et al.*, 2012b; Mowlick *et al.*, 2013; Hewavitharana *et al.*, 2014; McCarty *et al.*, 2014), composted broiler litter (Hewavitharana *et al.*, 2014; Di Gioia *et al.*, 2017), and residues of different cover crops. Amendment rates with these materials have varied between 3 to 90 t ha⁻¹ (Shrestha *et al.*, 2016).

ASD has not yet been practically applied in Italy, and there is still a need to adapt this soil disinfestation method to the cultural and environmental conditions of this country. The choice of effective, cheap and easily available C-sources is of particular importance. Although a number of studies have reported effects of ASD on soil-borne pathogens of many crops, no studies have been carried out on leafy vegetables. These represent an intensive production system in many countries, and are particularly important in Italy, both in open fields and protected systems (Gullino *et al.*, 2019). There is no standardised ASD method to determine the best combination for soil-borne pathogen control, and several factors, such as the C-source, the rate of application, the treatment duration, the temperature under the plastic covers or the type of soil, can influence the effectiveness of this disease management method.

Rhizoctonia solani (Kühn), the soil-borne fungus that causes basal rot of a broad range of hosts, is one of the most important pathogens affecting lettuce production in Italy. This pathogen is also important in most countries where lettuce is grown (Blancard *et al.*, 2003; Barrière *et al.*, 2014; Gullino *et al.*, 2019).

The present study aimed to evaluate effects of ASD on the *R. solani*-lettuce pathosystem, under controlled conditions, and considering several factors. These included: i) different carbon sources; ii) different temperature regimes; and iii) two treatment durations. The effects on disease incidence and lettuce fresh weight were evaluated in two soil types considering two crop cycles planted in an ASD-treated soil, and in reference experimental controls with or without anaerobiotic conditions. Results obtained have been compared with those from tolclofos-methyl, the available fungicide for control of *R. solani* basal rot of lettuce.

MATERIALS AND METHODS

Experimental layout

Two trials were carried out at the Agroinnova Centre of Competence of the University of Torino, Grugliasco, under controlled conditions. ASD treatments were applied in four growth chambers and in two greenhouse compartments (64 m² each), to test the treatment efficacies against *R. solani*. Different carbon sources, including compost (Comp), *Diplotaxis tenuifolia* green manure (WR) and *Brassica carinata* pellets (BCp), and two types of soil (peat and sandy-loam) were used, at different temperatures (21, 26 or 31°C) and two durations (3 and 6 weeks) as the main experiment factors. The ASD treatments were carried out in plastic containers (capacity, 40 L; dimensions 50 × 40 × 20 cm), soil surface area (2,000 cm²) using two types of soil: i) a mixture (50:50 v:v) of a sandy loam soil (sand : silt : loam, 68.16 : 10.7 : 21.1; pH 7.9; organic matter 0.94%), and perlite (Perlite Italiana Agrilit 3); ii) a peat substrate (Tecno 2, 70% white peat and 30% clay; pH 6.1–6.5; N 110–190 mg L⁻¹; P₂O₅ 140–230 mg L⁻¹; K₂O 170–280 mg L⁻¹, Turco Silvestro terricci). The plastic containers were filled with 30 L of the treated or untreated soil distributed in a layer of 15 cm deep.

At the end of each ASD incubation period carried out in the growth chambers, the treated and untreated soil from each container was redistributed into four 12 L capacity plastic pots and kept in two compartments in a greenhouse at temperatures ranging from 27 to 30°C, and relative humidity (RH) of 70–80 %. The pots were arranged on benches in a completely randomized block design with one pot serving as a replicate, using four replicates per treatment. The experimental design of the trials is illustrated in Figure 1.

ASD treatment simulated under growth chamber conditions, and measurements

The ASD treatments started immediately, under controlled conditions in the growth chambers, after addition of the selected C-sources to the soil, and application of irrigation water at 17.0 L per container for the peat soil, or 7.0 L per container for the sandy loam soil. These irrigation rates were estimated by adding excess water to the respective soils and then allowing water to drain for 24 h, to simulate saturation conditions. The soil was then covered with standard polyethylene (PE) sheets (50 µm thick), immediately after the application of the different carbon sources and water, and the containers were moved to the growth chambers to start the ASD treatments at different temperatures.

The soils were incubated for 3 or 6 weeks at constant temperatures of 21°C (Trials 1 and 2), and at 26 (Trial 1) or 31°C (Trial 2) (Figure 1). These temperatures were selected to simulate typical soil conditions in the Mediterranean area during spring and summer (Tamietti and Garibaldi, 1987). The soil temperatures were monitored using a sensor data logging system (Digital Data Logger EM50; Decagon Devices). Redox potential values were taken manually each day (5 d per week, for 3 or 6 weeks), using an ORP/temp pen style meter (VWR International) at three positions in each container. The temperature and redox sensors were placed at the centre of each container at a depth of 10 cm.

The ORP values of the soil redox potential, expressed in mV, were converted to Eh mV, related to the redox potential of a standard hydrogen electrode, by adding 200 mV (Fiedler *et al.*, 2007). To calculate the cumulative soil anaerobic conditions, the absolute values of the difference between each redox value and the critical redox potential (CEh), calculated using the formula: 595 mV – (60 mV × soil pH measured at the end of the ASD treatment), were summed for redox values below CEh. Since the recorded data were the average daily redox potential values, the values obtained were multiplied by 24 h and converted into hourly units (mVh), then summed for each day. The cumulative soil anaerobic conditions (mVh) over the 3 or 6 weeks of the ASD treatments was then obtained (Rabenhorst and Castenson, 2005; Butler *et al.*, 2012 a; b).

Artificial infestation with the pathogen

One isolate (code AG2-L) of *R. solani*, obtained from affected lettuce, was used. The isolate was grown on PDA plates for 10 d. Flasks (1 L capacity), containing 300 g of sterilised wheat kernels, were inoculated with 5 mm diam. agar disks from *R. solani* PDA colonies, and were then maintained for 20 d at 23°C. Soils were infested with the pathogen by mixing 0.5 g L⁻¹ of the infested kernels, immediately before commencing the ASD treatments, using a total of 15 g of inoculum per container. The same amount of non-infested kernels was used for the non-inoculated experimental controls.

Carbon sources

Four carbon sources, selected among a number of different sources used in preliminary trials, were tested, and these are reported hereafter at selected field doses selected, according to the recommendations by Butler *et al.* (2014b) for moderate soil temperature:

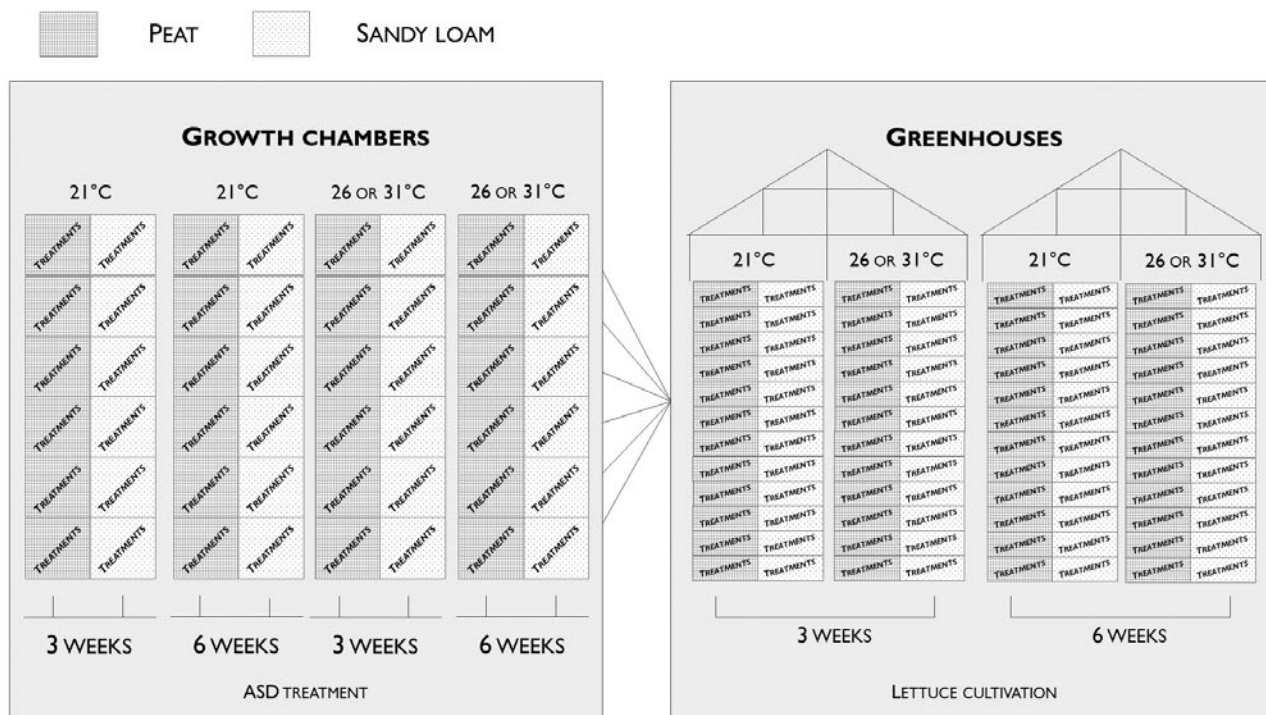


Figure 1. Experimental protocols utilized to assess the effect of anaerobic soil disinfestation (ASD) for the control of *Rhizoctonia solani* on lettuce. Assays were conducted in four growth chambers and in two greenhouse compartments during two trials carried out in peat and sandy-loam soils.

i) WR, wild rocket (*Diplotaxis tenuifolia*, C/N 6.9) applied as a green manure crop, at 10 g L⁻¹, equivalent to 15 t ha⁻¹ 15 cm depth, or at 20 g L⁻¹, equivalent to 30 t ha⁻¹;

ii) BCp, *Brassica carinata* defatted pellet ('Biofence': C/N 10.96, organic N 6%, P 2.2%, K 2%, organic C 52%: Triumph), mixed into the soil at 10 g L⁻¹, equivalent to 15 t ha⁻¹ 15 cm depth;

iii) Comp, compost prepared from green wastes in a dynamic industrial treatment system, and passed through a 20 mm sieve (Ant's Compost V, lot number N-2015, AgriNewTech: C/N 16.5, pH 6.58; total N 0.971%; total C 16%; P₂O₅ 0.57%; K₂O 1.35%), mixed into the soil at 10 g L⁻¹, equivalent to 15 t ha⁻¹ 15 cm depth.

Diplotaxis tenuifolia, 'Grazia', was sown at 1-1.2 g m⁻² in plastic pots filled with sterile blonde sphagnum peat, to produce wild rocket green manure. The pots were kept in a greenhouse at 24-26°C for 40-50 d (50% flowering). The plants were harvested, weighed, and cut into 1 to 3 cm pieces and incorporated into the soil, immediately before commencing the ASD treatment.

Experimental control treatments

Tolclofos-methyl TM (Rizolex, BASF Crop Protection, 50% a.i.) was used at 2 g m⁻² in both trials as a fun-

gicide experimental control, because this compound is effective for management of basal rot of lettuce, caused by *R. solani* (Sneh *et al.*, 1996). In Trial 2, the commercial formulation 'Biofence' based on *Brassica carinata* defatted pellets was applied without anaerobic conditions, at the same rate used for the ASD treatment, as reference control (BCp-No anaerobic control).

Two inoculated untreated controls, without any carbon source incorporated into the soil but with differences in the volume of water used, were prepared:

i) a standard untreated control (INT-Standard control): irrigated to field soil capacity;

ii) an INT-Anaerobic control: irrigated to exceed field capacity, to provide reducing conditions.

In the inoculated and non-inoculated untreated controls (INT and NINT) as well as for the BCp-No anaerobic control, water equivalent to the moisture capacity of the peat soil (8.0 L per container) and sandy loam soil (5.0 L per container) was used. Two non-inoculated control pots, which received sterile wheat kernels, under an anaerobic condition (NINT- Anaerobic control) and under a standard condition (NINT-Standard control), were also included.

Both INT and NINT standard and anaerobic controls as well TM and BCp- No anaerobic control were

each covered with a transparent polyethylene film (50 μm thick) for the 3 or 6 weeks time treatments.

Effects of ASD treatment in greenhouse trials

At the end of the ASD treatments carried out in growth chambers, the soil was transferred into 12 L capacity pots and was then aerated for 1 week before planting lettuce 'Elisa' (20 plants per pot). Lettuce seedlings were transplanted 20 to 25 day after sowing into the treated and untreated soil in two subsequent crop cycles.

Fertilizer equivalent to 20 kg ha⁻¹ (N 18; P 18; K 18 + 2 MgO, Osmoform) was applied to the soil surfaces at the end of the first cycle in both trials.

Disease incidence (percent dead plants) was evaluated each week by counting the collapsed and dead plants that showed brown, sunken lesions and rotting at the base of the crowns. Dead plants were removed and randomly selected to perform pathogen isolation. The plants were removed from the soil at the end of each cropping cycle in both trials, at the full maturity stage, to evaluate the final *Rhizoctonia* basal rot incidence, and the fresh weights of all healthy plants were determined.

Data analyses

The experiments were carried out and analysed as two independent trials, at the end of the first and second crop cycles (Tables 1, 2 and 3). Data of mean accumulated soil anaerobic conditions values were subjected to analysis of variance (ANOVA) in the SPSS software, with means separation based Tukey's test ($P \leq 0.05$). Disease inci-

dence (*DI*) and fresh weights (*FW*) of the healthy lettuce plants were subjected to ANOVA using the univariate procedure in the SPSS software 25.0 statistical package. Levene's test was used to test the homogeneity of variances. The *DI* data, expressed as a percentage of affected plants, were arc-sin-transformed to stabilise the variances and normalise their distribution. The effect of each carbon source, temperature, ASD duration (3 or 6 weeks) for two types of soil (peat or sandy-loam), and their interactions for both crop cycles, were evaluated as main factors. When the effects of the tested factors were significant ($P \leq 0.05$) and interactions were observed among the considered factors (Table 3), one-way ANOVA was carried out to evaluate the combined effects of the involved factors on the percent disease incidence and plant fresh weights. The means were separated using Tukey's test ($P \leq 0.05$) when the multiple comparisons of the considered factors were shown to be significantly related.

RESULTS

Impacts of the soil treatments on soil parameters

On the basis of the oxidation-reduction potential (ORP), the reduced conditions in the ASD treatments of the peat and sandy-loam soils were generally reached within 4 to 5 d for all the tested temperatures. The lowest cumulative soil anaerobic conditions indicated by the redox potential were observed in the no C source control (INT-Standard control) in the sandy-loam soil at 21°C and 26°C for both 3 and 6 weeks, and in the peat soil at 21°C for 3 weeks, while increased values were observed in the INT- control under anaerobic conditions for 6 weeks at 31°C (85,766 mVh) (Table 2). The greatest levels of cumulative anaerobic conditions were observed in the peat soil treated with WR at 15 g ha⁻¹ for 6 weeks at 26°C in Trial 1 (211,935 mVh), and at 30°C for 6 weeks in Trial 2 (180,162 mVh at 15 t ha⁻¹ and 184,683 mVh at 30 t ha⁻¹). The ASD based-WR treatment at 15 ha⁻¹ in the sandy-loam soil at 31°C (trial 2) provided cumulative anaerobic conditions of 82,788 mVh, and at 30 t ha⁻¹, 119,066 mVh. At 21°C cumulative anaerobic conditions were 43,947 mVh from 15 t ha⁻¹ and 72,440 mVh from 30 t ha⁻¹. The cumulative anaerobic conditions calculated for 6 weeks from the ASD based-BCp treatment were different among the trials, and the temperature conditions tested (from 106,054 to 176,736 mVh in the peat soil and from 86,950 to 112,968 mVh in the sandy-loam soil), with significantly lower mVh values for 3 weeks of ASD duration. Three weeks of ASD based-Comp in the peat soil at 21°C provided cumulative anaerobic conditions of the soil between 1,206 and 4,803 mVh and of

Table 1. List and dates of the different operations carried out in greenhouse experiments.

Operations	Trial 1	Trial 2
Artificial soil infestation with <i>Rhizoctonia solani</i>	17/01/16	31/05/16
Start of the ASD treatments lasting 6 weeks	18/01/16	1/06/16
Start of the ASD treatments lasting 3 weeks	8/02/16	22/06/16
Lettuce transplant (Cycle I)	11/03/16	20/07/16
Disease assessments	4/04/16	10/08/16
Lettuce fresh weights determined (Cycle I)	4/04/16	10/08/16
Lettuce transplant (Cycle II)	8/04/16	11/08/16
Disease assessments	10/5/16	20/09/16
Lettuce fresh weights determined (end of Cycles I and II)	10/05/16	20/09/16

Table 2. Average soil pH and the mean cumulative soil redox potential (mVh) indicating anaerobic conditions, for the peat and sandy-loam soils after different soil treatments. Standard errors are indicated.

Carbon source ^a , dosage	ASD	Treatment duration (weeks)	Trial 1				Trial 2							
			21°C		26°C		21°C		31°C					
			pH	mVh	pH	mVh	pH	mVh	pH	mVh				
<i>Peat soil</i>														
Comp, 15 t ha ⁻¹	+	3	6.6	1,206 ± 603	b ^c	6.7	12,532 ± 889	de	6.6	4,803 ± 109	e	6.6	13,667 ± 837	de
BCp, 15 t ha ⁻¹	+	3	7.1	41,235 ± 3,405	ab	7.2	34,666 ± 1,987	cd	6.8	27,708 ± 970	c	6.9	50,409 ± 1,705	c
WR, 15 t ha ⁻¹	+	3	7.0	25,330 ± 7,491	ab	7.0	33,646 ± 6,014	cd	6.4	30,916 ± 371	c	6.6	30,696 ± 1,242	cd
WR, 30 t ha ⁻¹	+	3	- ^b	-	-	-	-	-	6.3	31,373 ± 1,028	c	6.7	47,706 ± 3,148	c
TM, 2 gm ⁻²	-	3	6.6	0 ± 0	b	6.8	0 ± 0	e	6.8	0 ± 0	e	6.8	0 ± 0	e
BCp, 15 t ha ⁻¹ - No anaerobic control	-	3	-	-	-	-	-	-	6.8	0 ± 0	e	6.9	0 ± 0	e
INT- Anaerobic control	+	3	6.8	650 ± 620	b	6.6	4,811 ± 1,941	e	6.5	1685 ± 822	e	6.5	5,267 ± 382	e
INT- Standard control	-	3	6.4	0 ± 0	b	5.9	0 ± 0	e	6.7	0 ± 0	e	6.7	0 ± 0	e
C, 15 t ha ⁻¹	+	6	6.6	25,972 ± 1,482	ab	6.6	46,495 ± 5,078	c	6.6	18,205 ± 1,267	d	6.5	95,550 ± 4,349	b
BCp, 15 t ha ⁻¹	+	6	6.9	106,054 ± 4,167	a	7.1	145,895 ± 1,0183	b	6.9	135,800 ± 1,094	a	6.7	176,736 ± 1,094	a
WR, 15 t ha ⁻¹	+	6	6.7	89,537 ± 3,866	a	6.9	211,935 ± 8,192	a	6.6	92,639 ± 5,773	b	6.6	180,162 ± 4,002	a
WR, 30 t ha ⁻¹	+	6	-	-	-	-	-	-	6.7	101,487 ± 2,181	b	6.5	184,683 ± 7,705	a
TM, 2 gm ⁻²	-	6	6.7	0 ± 0	b	6.6	0 ± 0	e	6.8	0 ± 0	e	6.6	0 ± 0	e
BCp, 15 t ha ⁻¹ - No anaerobic control	-	6	6.6	0 ± 0	b	6.7	0 ± 0	e	6.9	0 ± 0	e	6.7	0 ± 0	e
INT-Anaerobic control	+	6	6.7	21,770 ± 650	ab	6.5	21,107 ± 1,049	de	6.5	23,949 ± 1,562	cd	6.5	85,766 ± 5,795	b
INT-Standard control	-	6	6.3	0 ± 0	b	6.1	0 ± 0	e	6.7	0 ± 0	e	6.6	0 ± 0	e
<i>Sandy-loam soil</i>														
Comp, 15 t ha ⁻¹	+	3	7.8	1,017 ± 709	b	7.3	2,606 ± 1,598	c	7.8	1,914 ± 475	d	7.9	2,805 ± 1,426	c
BCp, 15 t ha ⁻¹	+	3	7.4	10,822 ± 4,962	b	7.3	23,579 ± 2,335	bc	7.5	11,315 ± 2,507	cd	7.8	20,403 ± 3,342	c
WR, 15 t ha ⁻¹	+	3	7.0	10,980 ± 1,371	b	7.3	22,110 ± 971	bc	7.7	10,937 ± 4,042	cd	7.9	23,491 ± 2,790	c
WR, 30 t ha ⁻¹	+	3	-	-	-	-	-	-	7.6	12,474 ± 4,305	cd	7.6	25,470 ± 2,221	c
TM, 2 gm ⁻²	-	3	7.7	0 ± 0	b	7.8	0 ± 0	c	7.1	0 ± 0	d	7.8	0 ± 0	c
BCp, 15 t ha ⁻¹ - No anaerobic control	-	3	-	-	-	-	-	-	7.8	0 ± 0	d	7.9	0 ± 0	c
INT-Anaerobic control	+	3	7.8	583 ± 333	b	7.8	1,158 ± 657	c	7.9	36 ± 36	d	7.9	1,272 ± 756	c
INT-Standard control	-	3	7.8	0 ± 0	b	7.8	0 ± 0	c	7.9	0 ± 0	d	7.8	0 ± 0	c
Comp, 15 t ha ⁻¹	+	6	7.1	8,632 ± 1,495	b	7.1	15,516 ± 4,228	c	7.9	15,485 ± 1,842	cd	7.5	31,817 ± 1,593	bc
BCp, 15 t ha ⁻¹	+	6	7.2	86,950 ± 3,994	a	7.0	112,968 ± 5,599	a	7.8	109,688 ± 7,260	a	7.6	88,945 ± 3,893	a
WR, 15 t ha ⁻¹	+	6	7.4	78,396 ± 5,631	a	7.2	101,994 ± 4,806	a	7.9	43,947 ± 16,737	bc	7.7	82,788 ± 796	ab
WR, 30 t ha ⁻¹	+	6	-	-	-	-	-	-	7.8	72,440 ± 25,320	ab	7.7	119,066 ± 13,879	a
TM, 2 gm ⁻²	-	6	8	0 ± 0	b	7.7	0 ± 0	c	7.8	0 ± 0	d	7.8	0 ± 0	c
BCp, 15 t ha ⁻¹ - No anaerobic control	-	6	8	0 ± 0	b	7.5	0 ± 0	c	7.7	0 ± 0	d	8.0	0 ± 0	c
INT-Anaerobic control	+	6	7.4	353 ± 353	b	7.5	1916 ± 1,057	c	7.9	2,311 ± 643	cd	7.9	2,372 ± 754	c
INT-Standard control	-	6	-	0 ± 0	b	7.7	0 ± 0	c	7.8	0 ± 0	d	7.7	0 ± 0	c

^a Comp: Compost; BCp: *Brassica carinata* pellet; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT-anaerobic control: Inoculated untreated control with anaerobic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions.

^b - indicates not tested.

^c Means accompanied by the same letter are not significantly different ($P \leq 0.05$), Tukey test.

between 1,017 and 1,914 mVh in the sandy-loam soil. The cumulative anaerobic conditions of the ASD based-Comp were increased in the peat soil at 26°C and 31°C

after 3 weeks to, respectively, 12,532 mVh and 13,667 mVh, while the cumulative values in the sandy loam soil after 3 weeks from this treatment at 26°C and 31°C

Table 4. Interaction between ASD duration (weeks) and temperature (°C) in the peat or sandy-loam soils artificially infested with *Rhizoctonia solani*, for Trials 1 and 2. The data are expressed as A) mean % of lettuce plants with basal rot at the end of the I and II crop cycles B), and mean fresh weights (g pot⁻¹) at the end of the I and II crop cycles. All data represent results from trials 1 and 2.

ASD duration (weeks) × temperature (°C)	Peat soil				Sandy-loam soil			
	Mean % affected lettuce plants		Mean plant fresh weight (g pot ⁻¹)		Mean % affected lettuce plants		Mean plant fresh weight (g pot ⁻¹)	
	Cycle I	Cycle II	Cycle I	Cycle II	Cycle I	Cycle II	Cycle I	Cycle II
<i>Trial 1</i>								
3 weeks at 21°C	54.4 ±8.2 c ^a	50.0 ±6.6 c	95.7 ±24.3 bc	60.4 ±11.0 a	38.8 ±5.7 a	24.8 ±3.2 a	49.9 ±7.4 b	190.4 ±9.8 a
3 weeks at 26°C	50.2 ±7.4 b	39.7 ±5.7 ab	67.7 ±14.8 c	68.7 ±10.0 a	39.5 ±6.3 a	37.0 ±3.5 bc	52.8 ±8.6 b	131.7 ±8.9 c
6 weeks at 21°C	44.4 ±6.9 b	43.4 ±6.3 bc	117.5 ±20.0 ab	74.1 ±14.1 a	47.8 ±6.8 b	29.8 ±3.4 ab	44.9 ±8.0 b	161.4 ±7.5 b
6 weeks at 26°C	33.4 ±5.7 a	33.8 ±5.2 a	140.7 ±25.5 a	58.6 ±6.7 a	43.6 ±7.0 ab	41.7 ±4.3 c	65.4 ±10.8 a	151.7 ±9.2 bc
<i>Trial 2</i>								
3 weeks at 21°C	40.4 ±3.9 b	39.4 ±3.7 b	90.4 ±33.9 b	127.6 ±19.7 c	18.7 ±1.9 a	20.8 ±2.0 a	77.1 ±4.3 a	13.4 ±6.2 a
3 weeks at 31°C	14.6 ±2.0 a	18.9 ±2.3 a	74.0 ±24.2 b	150.7 ±20.2 c	18.5 ±2.2 a	20.3 ±2.1 a	68.0 ±7.4 ab	12.7 ±5.8 a
6 weeks at 21°C	34.0 ±4.2 b	27.0 ±3.2 b	114.6 ±18.0 a	356.5 ±26.5 a	21.1 ±2.3 ab	21.9 ±2.3 ab	60.3 ±6.1 b	10.2 ±4.6 a
6 weeks at 31°C	21.5 ±3.4 a	11.5 ±2.4 a	119.5 ±23.4 a	251.7 ±25.3 b	22.5 ±2.8 b	24.0 ±2.9 b	79.9 ±8.3 a	16.0 ±7.5 a

^a Means in each column accompanied by the same letter are not significantly different for Tukey test ($P \leq 0.05$) for cycle I and cycle II in each trial. Standard errors are also indicated.

Table 5A. Mean percentages of lettuce plants affected by basal rot after application of different ASD soil treatments, carried out for 3 or 6 weeks, and at 21 or 26 °C in peat artificially infested with *Rhizoctonia solani*, at the end of the first and second crop cycles (Trial 1).

Carbon source ^a , dosage	ASD	Treatment duration (weeks)	Cycle I				Cycle II			
			21°C	E% ^c	26°C	E%	21°C	E%	26°C	E%
Comp, 15 t ha ⁻¹	+	3	90.0±0.0 d ^b	0.0	76.5±4.9 ef	6.4	49.3±0.8 b-d ^a	45.2	33.9±1.5 bc	52.1
BCp, 15 t ha ⁻¹	+	3	28.3±1.0 b	68.6	32.3±4.7 bc	60.5	54.8±11.8 b-d	39.1	45.0±3.1 c	36.4
WR, 15 t ha ⁻¹	+	3	85.4±4.6 d	5.1	71.2±3.5 d-f	12.9	56.4±5.0 b-d	37.3	40.6±4.5 bc	42.7
TM, 2 g m ⁻²	-	3	22.6±1.7 b	74.9	15.7±1.6 ab	80.8	30.4±4.2 b	66.2	29.6±5.9 bc	58.2
INT-Anaerobic control	+	3	90.0±0.0 d	0.0	53.1±2.8 d-e	35.0	72.0±6.1 de	20.0	66.1±8.4 de	6.6
INT-Standard control	-	3	90.0±0.0 d	0.0	81.7±8.3 f	0.0	90.0±0.0 e	0.0	70.8±2.3 e	0.0
NINT-Anaerobic control	+	3	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0
NINT-Standard control	-	3	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0
Comp, 15 t ha ⁻¹	+	6	61.8±1.7 c	28.8	27.1±3.2 bc	67.0	42.0±5.2 b	43.1	30.7±6.7 bc	54.5
BCp, 15 t ha ⁻¹	+	6	30.5±3.4 b	64.9	33.4±5.2 bc	59.4	38.0±6.4 bc	48.5	44.2±5.8 c	34.4
WR, 15 t ha ⁻¹	+	6	56.0±7.9 c	35.5	46.6±4.6 cd	43.3	42.7±4.6 bc	42.1	37.7±1.9 bc	44.1
TM, 2 g m ⁻²	-	6	15.6±5.9 ab	82.0	19.5±1.1 ab	76.3	31.5±2.1 b	57.3	23.6±1.9 b	65.0
INT-Anaerobic control	+	6	62.2±6.3 c	28.3	47.9±2.0 cd	41.7	71.3±6.7 de	3.4	48.0±4.3 cd	28.8
INT-Standard control	-	6	86.8±3.2 d	0.0	82.2±4.7 f	0.0	73.8±6.4 de	0.0	67.4±1.7 de	0.0
NINT-Anaerobic control	+	6	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0
NINT-Standard control	-	6	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0

^a Comp: Compost; BCp: *Brassica carinata* pellet with anaerobic conditions; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT-Anaerobic control: Inoculated untreated control with anaerobic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions; NINT-Anaerobic control: Non-inoculated untreated control with anaerobic conditions; NINT-Standard control: Non-inoculated untreated control without anaerobic conditions.

^b Means in each column accompanied by the same letter are not significantly different ($P \leq 0.05$), according to Tukey's test. Standard errors are also indicated.

^c E%: Disease reduction compared to the INT-Standard controls carried out for 3 or 6 weeks.

Table 5B. Mean percentages of lettuce plants affected by basal rot after application of different ASD soil treatments, carried out for 3 or 6 weeks, and at 21 or 31°C in peat artificially infested with *Rhizoctonia solani*, at the end of the first and second crop cycles (Trial 2).

Carbon source ^a , dosage	Treatment		Cycle I				Cycle II			
	ASD	duration (weeks)	21°C	E% ^c	26°C	E%	21°C	E%	26°C	E%
Comp, 15 t ha ⁻¹	+	3	86.8±3.2 cd ^b	3.6	39.0±4.4 c-e	21.1	38.5±0.7 c-g	27.5	67.0±8.4 b-e	25.6
BCp, 15 t ha ⁻¹	+	3	45.3±2.0 ab	49.7	23.71±1.1 b-e	52.0	36.14±7.9 c-g	32.0	63.1±15.5 b-e	29.9
WR, 15 t ha ⁻¹	+	3	80.1±6.3 b-d	11.0	38.2±5.1 c-e	22.7	40.6±4.5 d-g	23.5	42.8±4.2 a-c	52.4
WR, 30 t ha ⁻¹	+	3	84.3±5.7 b-d	6.3	30.11±0.5 b-e	39.1	43.6±3.8 d-g	17.9	59.2±15.2 a-d	34.2
TM, 2 gm ⁻²	-	3	19.7±4.2 a	78.1	14.6±5.4 a-c	70.4	13.4±21.0 a-c	74.8	0.0±0.0 a	100.0
BCp, 15 t ha ⁻¹ - No anaerobic control	-	3	90.0±0.0 d	0.0	38.8±6.1 c-e	21.5	42.8±2.2 d-g	19.4	67.5±13.9 b-e	25.0
INT- Anaerobic control	+	3	90.0±0.0 d	0.0	47.9±1.7 de	3.0	54.7±3.6 g	0.0	82.5±7.5 de	8.3
INT- Standard control	-	3	90.0±0.0 d	0.0	49.4±1.9 de	0.0	53.1±2.6 fg	0.0	90.0±0.0 e	0.0
NINT- Anaerobic control	+	3	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0
Comp, 15 t ha ⁻¹	+	6	57.3±±4.7 b	28.1	40.6±2.8 c-e	20.1	28.2±1.8 c-e	31.6	31.6±0.9 a-c	48.2
BCp, 15 t ha ⁻¹	+	6	53.8±2.9 b	32.5	10.7±7.1 ab	78.9	3.2±3.2 ab	92.2	33.9±1.5 a-c	44.4
WR, 15 t ha ⁻¹	+	6	71.4±6.9 b-d	10.4	8.3±8.3 a	83.7	21.3±2.9 a-e	48.3	28.9±5.4 a-c	52.6
WR, 30 t ha ⁻¹	+	6	64.1±10.5 bc	19.6	11.4±6.6 ab	77.6	16.8±2.4 a-c	59.2	30.3±8.6 a-c	50.3
TM, 2 gm ⁻²	-	6	21.3±2.2 a	73.3	17.9±6.3 a-e	64.8	8.6±3.0 ab	79.1	24.4±5.2 ab	60.0
BCp, 15 t ha ⁻¹ - No anaerobic control	-	6	69.3±14.2 b-d	13.0	36.1±2.8 c-e	28.9	29.5±3.9 c-e	28.4	68.9±10.6 c-e	-13.0
INT- Anaerobic control	+	6	70.58.9 b-d	11.5	39.3±2.4 de	22.6	32.1±4.4 c-f	22.1	63.1±13.3 b-e	-3.4
INT- Standard control	-	6	79.76.0 b-d	0.0	50.8±1.7 e	0.0	41.2±2.4 d-g	0.0	61.0±2.4 b-e	0.0
NINT- Anaerobic control	+	6	0.00.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0

^a Comp: Compost; BCp: *Brassica carinata* pellet with or without anaerobic conditions; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT-Anaerobic control: Inoculated untreated control with anaerobiotic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions; NINT-Anaerobic control: Non inoculated untreated control with anaerobic conditions.

^b Means in each column accompanied by a common letter are not significantly different ($P \leq 0.05$), according to Tukey's test. Standard errors are also reported.

^c E%: Disease reduction compared to the INT-Standard controls carried out for 3 or 6 weeks.

slight reduction in ASD based-BCp efficacy occurred at the end of the second cycle carried out in the same soil (34–39% efficacy) (Table 5A). The same trend was found in the Trial 2 when ASD based-BCp was applied for 3 weeks at 21°C or 31°C (50–51% efficacy), with an increase in efficacy to 79% when incubated at 31°C for 6 weeks at the end of the first cycle. A positive effect of 6 weeks of ASD based-BCp also occurred in the second crop cycle (Table 5B). BCp applied at 15 t ha⁻¹ without anaerobic conditions and tested in Trial 2, provided no statistically significant advantages when applied to the infested peat soil for any of the tested conditions (Tables 5B).

WR used as green manure and mixed with the peat soil was not effective against basal rot when applied for 3 weeks at any of the tested temperatures in Trials 1 and 2 at the end of the first cycle (Tables 5A and 5B) at both the tested dosages. This treatment applied for 6 weeks at 26°C reduced ($P < 0.05$) the disease by 43%, and at 31°C by 78–84%, compared to the INT-Standard control,

without any effect of the dosage used.

The greatest efficacy of ASD based-Comp was observed in Trial 1 at 26°C for 6 weeks of incubation (67% efficacy), while the same carbon source did not provide any effect compared to the INT-Standard control in all the tested conditions at the first crop cycle in both trials (Tables 5A and 5B). ASD based-Comp gave increased efficacy against basal rot in the second crop cycle (32 to 54% efficacy), without any significant effects of temperature or duration of the treatment (Tables 5A and 5B).

At the end of the first cycle, the plants grown in the treated peat had generally reduced FW at 21°C and 26°C, and only the ASD based-Comp treatment applied for 6 weeks at 26°C improved ($P < 0.05$) the mean plant fresh weights, compared to the inoculated and untreated standard control (Table 6A). The lettuce plants grown in the same peat soil, previously treated with Comp and WR as C-sources for 3 or 6 weeks during the second crop cycle, at both temperatures, generally had greater

Table 6A. Mean fresh weight (g pot⁻¹) of lettuce plants after application of different ASD soil treatments, carried out for 3 or 6 weeks, and at 21 or 26 °C in peat artificially infested with *Rhizoctonia solani*, at the end of the first and second crop cycles (Trial 1).

Carbon source ^a , dosage	ASD	Treatment duration (weeks)	Cycle I				Cycle II			
			21°C		26°C		21°C		26°C	
Comp, 15 t ha ⁻¹	+	3	2.0±0.0	e ^b	18.0±7.6	c	197.3±5.3	a	166.1±25.2	a
BCp, 15 t ha ⁻¹	+	3	70.0±3.7	cd	93.3±12.4	bc	76.4±19.6	b-d	94.6±15.1	b-d
WR, 15 t ha ⁻¹	+	3	2.7±2.0	e	9.6±3.9	c	78.9±14.3	b-d	43.4±4.6	ef
TM, 2 gm ⁻²	-	3	67.4±9.2	cd	84.1±2.5	c	40.5±0.8	cd	35.3±2.0	f
INT- Anaerobic control	+	3	2.2±0.0	e	16.4±4.0	c	16.3±5.5	cd	29.3±13.0	f
INT- Standard control	-	3	2.6±0.0	e	2.4±0.0	c	3.0±2.0	d	17.0±4.5	f
NINT- Anaerobic control	+	3	387.6±25.6	a	232.8±27.3	ab	14.0±1.6	cd	33.0±5.1	f
NINT- Standard control	-	3	238.1±19.3	b	153.3±24.3	ab	80.1±8.2	b-d	131.3±8.2	ab
Comp, 15 t ha ⁻¹	+	6	93.21±1.8	c	356.2±111.4	a	201.7±14.1	a	108.9±6.1	b
BCp, 15 t ha ⁻¹	+	6	52.21±1.4	c-e	143.6±17.8	bc	163.2±59.7	ab	35.9±2.6	f
WR, 15 t ha ⁻¹	+	6	96.22±3.9	cd	87.8±14.1	bc	93.7±13.6	b-d	105.4±8.6	b
TM, 2 gm ⁻²	-	6	98.7±24.7	cd	87.4±14.4	bc	42.3±3.7	cd	58.1±11.7	c-f
INT- Anaerobic control	+	6	27.1±8.4	de	42.4±14.2	c	16.7±6.4	cd	46.5±6.8	d-f
INT- Standard control	-	6	1.8±1.0	e	8.1±5.3	c	17.0±11.6	cd	11.7±1.3	f
NINT- Anaerobic control	+	6	210.8±7.4	b	254.3±48.4	ab	13.9±2.7	cd	15.8±2.0	f
NINT- Standard control	-	6	360.419.7	a	246.1±31.5	ab	44.3±4.4	cd	86.3±2.4	b-e

^a Comp: Compost; BCp: *Brassica carinata* pellet with anaerobic conditions; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT- Anaerobic control: Inoculated untreated control with anaerobic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions; NINT-Anaerobic control: Non-inoculated untreated control with anaerobic conditions; NINT-Standard control: Non-inoculated untreated control without anaerobic conditions.

^b Means in each column accompanied by the same letter are not significantly different ($P \leq 0.05$), according to Tukey's test. Standard errors are also indicated.

fresh weights than during cycle I. The greatest mean fresh weights reached in the second crop cycle were recorded from the BCp and WR (at 15 t ha⁻¹) carbon sources at 31°C for 6 weeks incubation (Table 6B).

The infested non-treated pots without anaerobiosis (INT-Standard control) had 69% to 79% of basal rot affected plants in the sandy-loam soil at the end of the first crop cycle in Trial 1 (Table 7A), and there was a slight reduction in disease incidence at the end of Trail 2 (32–44% of affected plants) (Table 7B). The control treatment based on TM reduced the incidence of affected plants to a range of 74 to 90% in the sandy-loam soil (Tables 7A and 7B). BCp applied under anaerobic conditions in the sandy-loam soil at 21°C, provided reduced disease (52% in Trial 1 and 72% in Trial 2) when carried out for 3 weeks, compared to INT-Standard control. The same carbon source, applied under anaerobic conditions generally gave more consistent disease reduction when applied at 26°C for both 3 and 6 weeks (52–63% efficacy), and complete disease control was provided by this treatment when it was applied for 6 weeks at 31°C (Table 7B). The efficacy of the ASD based-BCp was generally improved in the second crop cycle (Tables 7A and

7B). The BCp treatment applied without any anaerobic conditions in the sandy-loam soil and tested in Trial 2, reduced basal rot incidence, compared to the INT-Standard control, by 71 % at 31°C for 6 weeks and by 44% at 21°C for 3 weeks (Table 7B). The ASD treatment with WR as green manure applied to the sandy-loam soil at 21°C for 3 weeks generally partially reduced the percentage of affected plants (32–37% efficacy) and resulted in a greater efficacy (from 46 to 59%) at 31°C (Table 7B), with no significant difference between the two dosages in Trial 2. The efficacy of the ASD treatment with WR generally increased in the second crop cycle for all the tested conditions (Tables 7A and 7B). Inconsistent results were observed when the compost was mixed into the sandy-loam soil, with the greatest disease reduction (38–41% efficacy) at 21°C for 3 weeks of incubation (Tables 7A and 7B). Efficacy of the ASD treatment with compost was generally increased in the second crop cycle, compared to the first, with the greatest disease reduction in the sandy-loam soil previously treated for 3 weeks at 21°C (53% efficacy in Trial 1 and 66% efficacy in Trial 2).

Mean fresh weights of the lettuce plants grown in the ASD treated sandy-loam soil at the end of the first crop

Table 6B. Mean fresh weight (g pot⁻¹) of lettuce plants after application of different ASD soil treatments, carried out for 3 or 6 weeks, and at 21 or 31 °C in peat artificially infested with *Rhizoctonia solani*, at the end of the first and second crop cycles (Trial 2).

Carbon source ^a , dosage	Treatment		Cycle I				Cycle II			
	ASD	Duration (Weeks)	21°C		31°C		21°C		31°C	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Comp, 15 t ha ⁻¹	+	3	10.5±0.5	b ^b	111.9±11.6	de	63.4±14.5	c-f	208.0±4.6	c-g
BCp, 15 t ha ⁻¹	+	3	36.5±0.0	b	246.5±81.7	a-d	32.7±10.1	d-f	162.0±71.0	d-g
WR, 15 t ha ⁻¹	+	3	13.0±12.6	b	287.4±74.8	a-d	41.3±13.1	d-f	198.0±8.3	c-g
WR, 30 t ha ⁻¹	+	3	11.0±1.0	b	79.6±6.1	e	141.6±7.0	a-d	281.5±10.4	b-f
TM, 2 gm ⁻²	-	3	102.2±28.1	a	373.65±0.1	a-c	192.4±15.0	ab	305.4±107.4	b-e
BCp, 15 t ha ⁻¹ - No anaerobic control	-	3	10.3±0.3	b	97.4±31.1	de	97.9±9.2	b-f	47.5±4.8	fg
INT- Anaerobic control	+	3	10.0±0.0	b	69.6±23.7	e	13.8±1.9	f	42.7±24.7	g
INT- Standard control	-	3	12.3±12.3	b	73.2±13.1	e	4.0±0.0	f	47.4±25.0	fg
NINT- Anaerobic control	+	3	117.4±24.0	a	233.7±13.9	b-e	206.21±2.1	a	301±31.8	b-e
Comp, 15 t ha ⁻¹	+	6	28.4±10.6	b	99.9±14.5	de	132.0±3.8	a-e	172.5±25.0	c-g
BCp, 15 t ha ⁻¹	+	6	13.1±1.8	b	419.0±39.5	ab	195.2±7.5	ab	657.1±45.4	a
WR, 15 t ha ⁻¹	+	6	15.6±6.5	b	460.3±31.8	ab	124.81±1.9	b-e	405.4±98.7	bc
WR, 30 t ha ⁻¹	+	6	33.9±17.5	b	186.5±50.6	c-e	165.5±25.0	a-c	497.4±52.6	ab
TM, 2 gm ⁻²	-	6	96.4±20.0	a	376±55.6	a-c	99.1±9.1	b-f	334.5±31.7	b-d
BCp, 15 t ha ⁻¹ - No anaerobic control	-	6	16.9±4.0	b	113.5±5.4	de	12.5±3.4	f	294.2±42.6	b-e
INT- Anaerobic control	+	6	15.7±9.5	b	101.3±7.8	de	25.0±8.7	ef	73.8±8.6	e-g
INT- Standard control	-	6	29.2±4.0	b	70.51±7.7	e	23.0±3.0	ef	89.5±18.9	e-g
NINT- Anaerobic control	+	6	152.9±14.1	a	221±13.5	c-e	132.7±10.1	b-e	239.3±20.4	c-g

^a Comp: Compost; BCp: *Brassica carinata* pellet with or without anaerobic conditions; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT-Anaerobic control: Inoculated untreated control with anaerobiotic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions; NINT-Anaerobic control: Non inoculated untreated control with anaerobic conditions.

^b Means in each column accompanied by a common letter are not significantly different ($P \leq 0.05$), according to Tukey's test. Standard errors are also reported.

cycle were very low for all the treatments (Tables 8A and 8B). Moreover, there was a significant reduction in the mean lettuce fresh weights in the non-infested control (NINT-Anaerobic control) incubated for 6 weeks. By the end of the second crop cycle, lettuce plants generally had significantly greater fresh weights than observed after the first cycle. The greatest mean fresh weight was at 21°C using WR at 15 t ha⁻¹ for 3 weeks and BCp for 6 weeks (Table 8A). The lettuce plants of the second crop cycle grown in the same sandy-loam soil, previously treated at 31°C for 6 weeks using WR at both the tested dosages and BCp, generally had significantly greater mean fresh weights than the non-inoculated control plants under anaerobic conditions (Table 8B).

DISCUSSION

ASD has been proposed in several reports as a possible solution for soil disinfestation to control several plant pathogens, on different crops, using a variety of C-sources, under controlled conditions and in field

experiments (Momma *et al.*, 2006; Katase *et al.*, 2009; Butler *et al.*, 2012 a, b; Runia *et al.*, 2012; 2014; Roskopf *et al.*, 2015; Strauss and Kluepfel, 2015; Hewavitharana and Mazzola, 2016; Shrestha *et al.*, 2016; Shennan *et al.*, 2014; 2018). Since this methodology requires further testing before practical implementation, considerable efforts have aimed to improve ASD efficacy with emphasis on the optimizing factors such as carbon sources (Butler *et al.*, 2012a; b; 2014b; Hewavitharana *et al.*, 2014; Shrestha *et al.*, 2016; Rodríguez-Molina *et al.*, 2016; Serrano-Pérez *et al.*, 2017), duration of the incubation periods, and soil temperatures (Hewavitharana *et al.*, 2015; Shrestha *et al.*, 2016; Shennan *et al.*, 2014; 2018) for developing standard treatments against specific soil-borne pathogens. For example, Runia *et al.*, (2012; 2014) set up a controlled laboratory system to simulate ASD treatments in mesocosms to study biotic and abiotic changes over time against *Verticillium dahliae* and *Globodera pallida*. A similar approach has also been used for other pathosystems, including *Phytophthora nicotianae*-pepper (Serrano-Pérez *et al.*, 2017) and *Verticillium dahliae*-strawberry (Shennan *et al.*, 2018).

Table 7A. Mean percentages of lettuce plants affected by basal rot after application of different ASD soil treatments, carried out for 3 or 6 weeks, and at 21 or 26 °C in sandy-loam artificially infested with *Rhizoctonia solani*, at the end of the first and second crop cycles (Trial 1).

Carbon source ^a , dosage	Treatment		Cycle I				Cycle II			
	ASD	Duration (Weeks)	21°C	E% ^c	26°C	E%	21°C	E%	26°C	E%
Comp, 15 t ha ⁻¹	+	3	45.0±1.2 cd ^b	41.2	47.3±4.4 de	34.5	19.9±3.6 bc	66.0	35.36±3.3 b-d	27.8
BCp, 15 t ha ⁻¹	+	3	37.0±1.9 c	51.6	27.1±3.2 b-d	62.5	33.2±1.3 cd	43.3	42.86±3.6 c-e	55.5
WR, 15 t ha ⁻¹	+	3	48.6±2.2 c-e	36.5	58.3±6.4 ef	19.3	27.8±3.7 bc	52.6	52.74±5.2 de	46.3
TM, 2 gm ⁻²	-	3	15.7±1.6 b	79.5	16.8±2.4 a-c	76.7	16.8±2.4 b	71.3	20.41±7.5 a-c	74.0
INT- Anaerobic control	+	3	67.4±1.7 fg	11.9	59.4±2.9 ef	17.7	46.5±3.9 d-f	20.6	56.18±2.7 de	19.9
INT- Standard control	-	3	76.5±4.9 g	0.0	72.2±6.4 f	0.0	58.6±2.9 f	0.0	54.68±3.0 de	0.0
NINT- Anaerobic control	+	3	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	4.61±4.6 a	100.0
NINT- Standard control	-	3	0.0±0.0 a	100.0	0.0±0.0 a	100	0.0±0.0 a	100.0	0.0±0.0 a	100.0
Comp, 15 t ha ⁻¹	+	6	57.9±3.6 d-f	16.1	42.1±4.1 de	46.6	34.6±2.8 cd	41.8	38.23±4.0 b-d	17.6
BCp, 15 t ha ⁻¹	+	6	49.4±3.9 c-e	28.4	36.6±5.0 cd	53.6	35.1±5.4 c-e	41.0	52.6±11.2 de	100.0
WR, 15 t ha ⁻¹	+	6	60.3±3.1 ef	12.6	78.8±6.7 f	0.1	34.7±0.9 c-e	41.7	56.73±5.14 de	49.9
TM, 2 gm ⁻²	-	6	7.8±4.7 ab	88.7	7.8±4.7 ab	90.1	15.7±1.6 ab	73.6	17.89±6.3 ab	84.6
INT- Anaerobic control	+	6	58.2±4.5 d-f	15.7	61.4±4.3 ef	22.2	50.9±6.9 ef	14.5	57.97±4.0 de	14.0
INT- Standard control	-	6	69.0±3.6 fg	0.0	78.9±3.9 f	0.0	59.5±3.4 f	0.0	64.7±2.9 e	0.0
NINT- Anaerobic control	+	6	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0
NINT- Standard control	-	6	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0

^a Comp: Compost; BCp: *Brassica carinata* pellet with anaerobic conditions; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT- Anaerobic control: Inoculated untreated control with anaerobic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions; NINT-Anaerobic control: Non-inoculated untreated control with anaerobic conditions; NINT-Standard control: Non-inoculated untreated control without anaerobic conditions.

^b Means in each column accompanied by the same letter are not significantly different ($P \leq 0.05$), according to Tukey's test. Standard errors are also indicated.

^c E%: Disease reduction compared to the INT-Standard controls carried out for 3 or 6 weeks.

In the present study, effects of ASD using *Brassicaceae* species, either as wild rocket green manure or *Brassica carinata* dry pellets, and compost as C-sources, were evaluated on the lettuce-*R. solani* pathosystem, considering effects on basal rot incidence and on lettuce productivity (fresh weights) in ASD treatments simulated in growth chambers. Our aim was to test the different ASD C-sources at one dosage equivalent to 15 t ha⁻¹, with the only exception being the double dosage tested for WR green manure. The commercial dosage of 3 t ha⁻¹ suggested for *B. carinata* pellets for biofumigation treatment was not considered. Our study was also carried out in two soil types, a sandy-loam alkaline soil and an acidic peat soil, at three temperatures (21, 26 or 31°C), and with two treatment durations (3 or 6 weeks). Impacts of these treatments on lettuce grown in the treated and untreated soil was also assessed over two consecutive crop cycles carried out in greenhouse.

Carbon sources, temperatures, incubation periods and their interactions affected the efficacy of the ASD treatments. Differences in efficacy depended on the soil type. *Rhizoctonia* basal rot was reduced at the higher

temperatures of 26°C or 31°C for 3 or 6 weeks in the peat soil, with resulting increased plant fresh weights, while generally in the sandy-loam soil, the greatest disease control was achieved with only 3 weeks of incubation, at all temperatures tested. In contrast, several studies have shown the impacts of temperature and ASD duration on pathogen survival, disease control and host yields in several pathosystems. Ebihara and Uematsu (2014) showed that *Fusarium oxysporum* f. sp. *fragariae*, *Phytophthora cactorum* and *Verticillium dahliae*, under anaerobic conditions, survived longer at 10°C, and were eradicated more rapidly at 40°C. Soil temperatures less than 30°C may be a critical factor in the effectiveness of ASD for reducing *Fusarium* wilt of strawberry (Muramoto *et al.*, 2015; Shrestha *et al.*, 2016).

Under the experimental conditions tested in the present study, different effects of the applied C-sources were observed. The impacts of *Brassica* crops and *Brassicaceae* seed meal have been shown to be effective bio-masses for ASD treatments to control soil-borne diseases caused by *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., and *Verticillium* spp. (Blok *et al.*, 2000; Messiha *et*

Table 7B. Mean percentages of lettuce plants affected by basal rot after application of different ASD soil treatments, carried out for 3 or 6 weeks, and at 21 or 31 °C in sandy-loam artificially infested with *Rhizoctonia solani*, at the end of the first and second crop cycles (Trial 2).

Carbon source ^a , dosage	Treatment		Cycle I				Cycle II			
	ASD	duration (weeks)	21°C	E% ^c	31°C	E%	21°C	E%	31°C	E%
Comp, 15 t ha ⁻¹	+	3	24.1±8.1 b-d ^b	38.2	32.0±4.3 c-e	27.8	15.4±2.5 a-c	52.3	32.0±4.3 e-g	22.7
BCp, 15 t ha ⁻¹	+	3	11.1±3.9 ab	71.5	19.7±4.2 b-d	55.5	11.1±3.9 a-c	65.6	9.0±3.1 a-c	78.2
WR, 15 t ha ⁻¹	+	3	26.4±2.5 b-d	32.3	23.8±1.0 b-d	46.3	21.8±4.3 b-e	32.5	22.8±0.0 c-f	44.9
WR, 30 t ha ⁻¹	+	3	24.0±4.2 b-d	38.5	18.1±2.0 bc	59.1	19.5±1.1 be	39.6	15.7±1.6 a-d	62.1
TM, 2 gm ⁻²	-	3	15.4±2.5 a-c	60.5	11.5±7.8 ab	74.0	9.7±3.2 ab	70.0	11.5±7.8 a-c	72.2
BCp, 15 t ha-1- No anaerobic control	-	3	21.8±4.3 b-d	44.1	30.6±3.0 c-e	30.9	26.4±2.5 c-f	18.3	9.7±3.2 a-c	76.6
INT- Anaerobic control	+	3	32.1±3.7 cd	17.7	35.5±0.8 de	19.9	32.1±3.7 ef	0.6	30.6±3.0 d-g	26.1
INT- Standard control	-	3	39.0±4.7 d	0.0	44.3±2.5 e	0.0	32.3±2.3 ef	0.0	41.4±1.4 f	0.0
NINT- Anaerobic control	+	3	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0
Comp, 15 t ha ⁻¹	+	6	32.3±2.3 cd	1.5	34.7±2.0 ce	17.6	31.5±2.1 ef	19.2	35.5±0.8 e-g	15.7
BCp, 15 t ha ⁻¹	+	6	17.6±8.2 a-c	46.3	0.0±0.0 a	100.0	11.1±3.9 a-c	72.5	0.0±0.0 a	100.0
WR, 15 t ha ⁻¹	+	6	15.7±1.6 a-c	52.1	21.1±3.4 b-d	49.9	15.7±1.6 a-d	59.7	20.2±2.9 b-e	52.0
WR, 30 t ha ⁻¹	+	6	17.7±3.2 a-c	46.0	30.1±4.7 ce	28.5	17.7±3.2 b-e	54.6	16.5±2.7 a-d	60.8
TM, 2 gm ⁻²	-	6	11.1±3.9 ab	66.2	6.5±3.7 ab	84.6	11.1±3.9 a-c	71.5	20.5±2.1 b-e	51.3
BCp, 15 t ha-1- No anaerobic control	-	6	28.9±2.1 b-d	13.1	12.2±4.7 ab	71.0	32.8±4.6 ef	15.9	27.3±2.6 c-f	35.2
INT- Anaerobic control	+	6	31.5±2.1 b-d	4.0	36.2±2.2 de	14.0	31.5±2.1 ef	19.2	36.2±3.0 fg	14.0
INT- Standard control	-	6	32.8±4.6 cd	0.0	42.1±3.2 e	0.0	39±4.7 f	0.0	42.1±3.2 g	0.0
NINT- Anaerobic control	+	6	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0	0.0±0.0 a	100.0

^a Comp: Compost; BCp: *Brassica carinata* pellet with or without anaerobic conditions; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT-Anaerobic control: Inoculated untreated control with anaerobiotic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions; NINT-Anaerobic control: Non inoculated untreated control with anaerobic conditions.

^b Means in each column accompanied by a common letter are not significantly different ($P \leq 0.05$), according to Tukey's test. Standard errors are also reported.

^c E%: Disease reduction compared to the INT-Standard controls carried out for 3 or 6 weeks.

al., 2007; Postma *et al.*, 2014; Hewavitharan and Mazzola, 2016; Mowlick *et al.*, 2012; 2013; Shennan *et al.*, 2014; 2018). In the present study, *Brassicaceae* seed meal without anaerobic condition reduced basal rot of lettuce but to a lesser extent than in the ASD treatments, which generally provided more consistent disease control for all the tested temperatures in both soils (between a range of 52 to 72% disease reduction). However, the tested rate of application was five times greater than that suggested for the 'Biofence' product (3 t ha⁻¹) for biofumigation treatment. The economic value of such a high rate application should be evaluated. However, Butler *et al.*, (2014 b) suggested that C source rates greater than 4 mg g⁻¹ of soil were required when soil temperatures during ASD treatments were low (15-25°C). Serrano-Pérez *et al.* (2017) confirmed that ASD under early spring conditions in Spain, using several C sources at 4 mg g⁻¹ of soil, was effective to control *P. nicotianae* disease in pepper. Nevertheless, in the present study effects on disease reduction and lettuce fresh

weights were not observed at the greatest WR green manure amount of 30 t ha⁻¹. The efficacy of ASD based-WR was generally greatest at the highest incubation temperature in the peat soil and at the lowest temperature of 21°C in sandy loam soil. MaCarty *et al.* (2014) showed that an ASD treatment based on a mixture of *Sinapis alba* and *Eruca sativa*, affected survival of *R. solani* at approx. 20°C, under accumulated anaerobic conditions of approx. 20,000 mVh. The accumulated soil anaerobic condition achieved in the present study during different treatments only partially explain the ASD efficacy. For instance, the greatest basal rot control was obtained from the *Brassicaceae* seed meal applied for 6 weeks in the sandy-loam soil at 31°C, and this treatment resulted in 88,954 mVh. Almost the same value of cumulative mVh, achieved at 21°C, was only partially effective in the control of *Rhizoctonia* basal rot (28-46% disease reduction), compared to the untreated controls. These were severely affected at the end of the first cultivation cycle. Also, the greatest efficacy of ASD using

Table 8A. Mean fresh weight (g pot⁻¹) of lettuce plants after application of different ASD soil treatments, carried out for 3 or 6 weeks, and at 21 or 26 °C in sandy-loam artificially infested with *Rhizoctonia solani*, at the end of the first and second crop cycles (Trial 1).

Carbon source ^a , dosage	ASD	Treatment duration (weeks)	Cycle I		Cycle II	
			21°C	26°C	21°C	26°C
Comp, 15 t ha ⁻¹	+	3	47.8±1.7 de ^b	20.8±1.9 ef	194.9±6.6 bc ^a	166.1±34.4 a-c
BCp, 15 t ha ⁻¹	+	3	18.1±0.3 ef	74.1±9.7 b-d	109.9±9.0 de	110.7±20.5 a-d
WR, 15 t ha ⁻¹	+	3	16.0±1.5 ef	7.4±1.3 f	303.5±5.4 a	67.4±19.4 d
TM, 2 gm ⁻²	-	3	67.2±6.6 cd	110.4±5.4 a-c	206.1±6.2 bc	167.2±29.2 a-c
INT- Anaerobic control	+	3	24.1±1.6 ef	9.9±1.4 f	170.9±18.5 b-d	74.5±15.9 cd
INT- Standard control	-	3	13.3±2.8 f	7.9±4.3 f	78.8±9.7 e	68.2±12.4 cd
NINT- Anaerobic control	+	3	117.5±12.4 ab	123.6±22.1 a-c	158.3±12.6 b-e	194.8±27.3 a-c
NINT- Standard control	-	3	105.2±2.3 ab	88.2±3.9 cd	300.8±15.6 a	204.9±38.5 ab
Comp, 15 t ha ⁻¹	+	6	14.7±2.5 f	13.8±0.6 f	170.2±27.1 b-d	151.5±16.6 a-c
BCp, 15 t ha ⁻¹	+	6	5.6±0.9 f	84.9±18.2 cd	236.2±24.3 ab	94.7±32.5 b-c
WR, 15 t ha ⁻¹	+	6	25.4±6.9 ef	4.8±2.8 f	136.2±19.0 c-e	83.8±23.6 b-d
TM, 2 gm ⁻²	-	6	87.9±10.5 a-c	155±11.7 a	152.2±14.3 c-e	203.6±24.9 ab
INT- Anaerobic control	+	6	2.5±1.1 f	18.5±2.2 f	88.2±19.6 e	101.8±27.3 a-d
INT- Standard control	-	6	17.6±9.5 ef	5.4±2.3 f	97.5±4.5 de	57.2±17.0 d
NINT- Anaerobic control	+	6	85.7±4.5 bc	137.8±6.8 ab	210.6±22.3 bc	168.5±12.9 a-c
NINT- Standard control	-	6	119.9±12.1 a	103.6±6.6 bc	200.1±13.2 bc	223.5±26.0 a

^a Comp: Compost; BCp: *Brassica carinata* pellet with anaerobic conditions; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT- Anaerobic control: Inoculated untreated control with anaerobic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions; NINT-Anaerobic control: Non-inoculated untreated control with anaerobic conditions; NINT-Standard control: Non-inoculated untreated control without anaerobic conditions.

^b Means in each column accompanied by the same letter are not significantly different ($P \leq 0.05$), according to Tukey's test. Standard errors are also indicated.

compost observed in the peat soil at 26°C for 6 weeks of incubation (67% efficacy) resulted in 46,495 mVh, while, at the higher cumulative value of 95,550 mVh, achieved using compost as C- source at 31°C, control of the disease was partial (20% disease reduction). Results from the present study are generally in agreement with those of Shennan *et al.* (2014; 2018), who provided evidence that accumulated soil anaerobic conditions of 50,000 mV h⁻¹ at 25°C, achieved using wheat bran in a sandy clay loam soil, was crucial for control *Verticillium dahliae* in strawberry plants, but the same anaerobic condition did not provide efficient inactivation of the pathogen at 15°C.

ASD efficacy is modulated by a complex mechanism, and since a low soil oxygen levels are prerequisites for pathogen inactivation (Runia *et al.*, 2014) for some pathogens such as *R. solani*, efficacy is closely related to the carbon source. This is possibly due to different volatile profiles resulting from ASD treated soil, and to microbiological changes (MaCarty *et al.*, 2014; Hewavitharana *et al.*, 2014; 2015). Several studies have also reported that inoculum inactivation in soil under different temperature and accumulated soil anaerobic conditions during

ASD is pathogen specific. Mowlick *et al.*, (2012; 2013) showed that the incubation temperature influenced the suppression of *F. oxysporum* f. sp. *spinaciae* to a great extent by stimulating the multiplication of the anaerobic bacteria related to the purely anaerobic clostridial groups in *Brassica*, oat and wheat bran ASD-treatments. The diversity in the clostridial groups was generally greatest in the *Brassica* and wheat bran ASD samples at 30°C, and the diversity was greatly reduced at 20°C in the *Brassica*-treated soil.

The present study has shown that ASD based on *Brassicaceae* and compost as C-sources applied in a sandy-loam soil, had impacts on the control of *R. solani* on lettuce, even at lower temperatures than those required for soil solarisation or biosolarisation (Gamiel, 2000), with a general improvement in disease control in the second crop cycle. For instance, the ASD based on compost was partially effective in reducing basal rot of lettuce at the end of the first crop cycle in both soils, while the greatest disease reductions of 53% and 66% were observed in the second crop cycle in the sandy-loam soil previously treated for 3 weeks at 21°C. The benefits of long-term compost treatments on differ-

Table 8B. Mean fresh weight (g pot⁻¹) of lettuce plants after application of different ASD soil treatments, carried out for 3 or 6 weeks, and at 21 or 31 °C in sandy-loam artificially infested with *Rhizoctonia solani*, at the end of the first and second crop cycles (Trial 2).

Carbon source ^a , dosage	Treatment		Cycle I			Cycle II				
	ASD	duration (weeks)	21°C	31°C		21°C	31°C			
Comp, 15 t ha ⁻¹	+	3	23.7±2.5	cd ^b	28.1±4.3	d-f	71.8±7.4	a-d	26.9±7.7	fg
BCp, 15 t ha ⁻¹	+	3	77.9±3.9	b	37.9±4.7	cd	94.7±9.0	ab	149.5±18.2	a
WR, 15 t ha ⁻¹	+	3	25.9±8.1	cd	26.3±1.0	d-f	77.9±6.4	a-c	56.0±5.0	d-f
WR, 30 t ha ⁻¹	+	3	26.0±4.2	cd	31.9±2.0	ce	89.0±5.8	a-c	74.0±5.6	c-e
TM, 2 gm ⁻²	-	3	38.2±2.3	c	43.6±3.7	c	64.1±12.4	a-e	64.3±20.1	c-f
BCp, 15 t ha ⁻¹ - No anaerobic control	-	3	34.6±2.5	cd	30.4±4.2	ce	96.1±23.2	ab	77.8±8.1	b-e
INT- Anaerobic control	+	3	17.9±3.7	cd	19.4±3.0	d-f	60.1±5.9	a-e	36.3±4.1	d-f
INT- No anaerobic control	-	3	17.7±2.3	cd	15.3±2.0	ef	48.1±12.8	b-e	23.6±3.2	g
NINT- Anaerobic control	+	3	116.3±8.3	a	109.8±5.6	b	92.2±8.4	ab	127.1±7.1	ab
Comp, 15 t ha ⁻¹	+	6	28.5±3.9	cd	15.7±2.5	ef	51.7±5.0	b-e	39.2±3.5	d-f
BCp, 15 t ha ⁻¹	+	6	67.3±3.6	b	50.0±0.0	c	83.4±8.7	a-c	150.4±12.9	a
WR, 15 t ha ⁻¹	+	6	34.4±1.6	cd	28.9±3.4	d-f	56.1±6.2	b-e	76.5±9.5	c-e
WR, 30 t ha ⁻¹	+	6	22.3±3.2	cd	19.9±4.7	d-f	99.3±2.1	ab	109.0±9.0	a-c
TM, 2 gm ⁻²	-	6	39.0±3.9	c	34.5±0.8	cd	36.5±8.2	c-e	77.1±9.3	b-e
BCp, 15 t ha ⁻¹ - No anaerobic control	-	6	32.4±8.2	cd	38.5±7.8	cd	111.6±22.1	a	49.4±5.6	d-f
INT- Anaerobic control	+	6	18.5±2.1	cd	13.8±2.2	e-f	22.8±1.6	de	35.8±11.7	d-f
INT- No anaerobic control	-	6	13.5±2.9	d	8.0±3.2	f	14.3±3.6	e	49.0±4.4	d-f
NINT- Anaerobic control	+	6	85.3±6.7	b	141.5±6.1	a	69.0±3.2	a-d	158.4±7.5	a

^a Comp: Compost; BCp: *Brassica carinata* pellet with or without anaerobic conditions; WR: *Diplotaxis* green manure; TM: Tolclofos methyl; INT-Anaerobic control: Inoculated untreated control with anaerobic conditions; INT-Standard control: Inoculated untreated control without anaerobic conditions; NINT-Anaerobic control: Non inoculated untreated control with anaerobic conditions.

^b Means in each column accompanied by a common letter are not significantly different ($P \leq 0.05$), according to Tukey's test. Standard errors are also reported.

ent pathosystems are well known (Abawi and Widmer, 2000; Chellemi, 2002; Gamliel, 2000; Lazarovits and Subbarao, 2010). However, a wide range of results have been achieved when compost has been used as organic amendments, including decreases and increases in soil-borne disease incidence and severity (Hoitink and Fahy, 1986; Abbasi *et al.*, 2002; Noble and Coventry, 2005; Bonanomi *et al.*, 2007; Pugliese *et al.*, 2011; 2015). Compost suppressiveness against *Rhizoctonia solani* in particular is known to be limited (Termorshuizen *et al.*, 2006; Pugliese *et al.*, 2015), and the use of composted manure for ASD has also been less effective than other carbon sources for controlling this pathogen (Hewavitharana and Mazzola, 2016).

Generally, the variation in efficacy of individual treatment combinations was limited in the second crop cycle, with positive effect on lettuce yields. However, the infection of plants by *Rhizoctonia solani* was generally less in the second crop cycle than in the first, and all the tested C-sources applied to both soils provided greater disease control in the second crop cycle, which resulted in increased lettuce yields.

Although it is well known that ASD treatments may have effects on soil fertility, by influencing different soil properties (McCarty *et al.*, 2014; Butler *et al.*, 2014b; Di Gioia *et al.*, 2017), they may also have negative effects on crop yields, due to low amounts of available N when large amounts of labile C are applied (Whitmore, 1996), or to phytotoxicity. Phytotoxic effects of ASD treatments could explain the low lettuce fresh weights measured after the first cycle, which may not only be attributable to differences in efficacy of the tested ASD treatments. High-N amendments in soils can lead to the production of ammonia, which is toxic to a wide range of pathogens and nematodes (Conn *et al.*, 2005; López-Robles *et al.*, 2013; Mazzola *et al.*, 2018), but also may have negative effects on plant development (Barker *et al.*, 1970). Readily decomposable amendments, under anaerobic conditions, can also favour the production of toxic organic acids which have been linked to the control of fungal, nematode, insect, and weed pests (Okazaki and Nose, 1986; Conn *et al.*, 2005; Momma *et al.*, 2006; Hestmark *et al.*, 2019). The possible phytotoxicity to lettuce from organic acid accumulation or salts during ASD treatments should be considered.

In the present study small increases in soil pH of 0.2 to 0.4 units was observed when *B. carinata* pellets were used. Increased suppressiveness of *Rhizoctonia* damping-off of sugar beet in a near neutral to alkaline soil, compared to acid soil, from use of dried peanut plant residues, has been reported, due to increased activity of specific antagonistic soil microorganisms (Watanabe *et al.*, 2011). Similar disease suppression effects have been obtained by amending soils with composts rich in cellulolytic and oligotrophic actinomycete antagonists (Tuitert *et al.*, 1998; Kasuya *et al.*, 2006; Ros *et al.*, 2006). Changes in the soil microbiome, induced by an ASD treatment in a carbon source dependent manner (Hewavitharan and Mazzola, 2016), can be persistent, and may result in long-term pathogen re-infestation (Goud *et al.*, 2004). Improved disease suppression and yields were observed for the ASD treatment-based compost in the second crop cycle, which is similar to other reports (Ros *et al.*, 2006; Hestmark *et al.*, 2019). Nevertheless, the increased lettuce fresh weights observed after ASD treatment in the second crop cycle may also be explained in part by improved nutritional status of the plants, as was observed by Butler *et al.*, (2014 b) and Di Gioia *et al.*, (2017).

No chemical or non-chemical methods used alone exhibit the same efficacy as some soil fumigants used in the past (Katan, 2017), so disease reduction offered by ASD studied here is promising. The potential of ASD for controlling *R. solani* incidence in lettuce merits further investigation, so that it can be adapted to different local conditions. The significant but only partial efficacy of ASD when *Brassica carinata* was applied as C-source does not justify the adoption of such treatment in the practice, but this approach integrated with other disease management methods could be worthwhile (Butler *et al.*, 2012 a). For sustainable agriculture, the use of compost or *Diplotaxis* green manure as other C-sources possible for evaluation of other green wastes. ASD based on *Diplotaxis* could be useful for managing crop residues in horticultural systems for ready-to-eat salad crops, where stringent quality requirements must be satisfied and crop damage thresholds of 5% make the products unmarketable.

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CONFLICT OF INTEREST

Massimo Pugliese declares he has a financial interest (shareholder) in AgriNewTech, the company that provided the compost tested in this research.

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