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Gaseous emissions from soil biodisinfestation by animal manure on a greenhouse pepper crop

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ABSTRACT

Soil solarisation together with the application of animal manure has been described as an alternative process for control of *Phytophthora capsici* root rot in pepper crops. A mixture of fresh sheep manure and dry chicken litter (SCM) and a semi-composted mixture of horse manure and chicken litter (HCM) were applied at 5.1 kg m^{-2} (dry weight) under plastic sheets to reduce *Phytophthora* inoculum survival rate and disease incidence. Non-solarised (C) and solarised (S) soils were used as control treatments. Mean NH_3 concentration increased in SCM during biodisinfestation process (14.8 mg NH_3 m^{-3}) compared with HCM (9.1 mg NH_3 m^{-3}), accounted for the higher organic N content and potential N mineralisation. The higher NH_3 concentration in SCM could have contributed to reduce the inoculum survival rate (30.6% and 75.0% in SCM and HCM plots, respectively). Inoculum survival rate was not reduced in S (94.4%) as temperature was below 33 °C throughout the experimental period. After biodisinfestation treatment, N_2O and CO_2 emissions tended to be higher in SCM, despite high spatial variability. Cumulative N_2O emissions were 1.31 and 0.42 g N_2O-N m^{-2} in SCM and HCM after 43 days. The larger N application and organic N mineralisation rate on fresh manure amended soils might have contributed to higher N_2O emissions during and after soil biodisinfestation by denitrification and nitrification, respectively. Cumulative CO_2 emission averaged 211.0 and 159.9 g CO_2-C m^{-2} in SCM and HCM, respectively. The soluble organic C, more abundant in fresh manure, might have favoured soil respiration in SCM. Disease incidence decreased in SCM and HCM plots (disease incidence, 2%–8%) in relation to solarised soils (42%) after 4 months. Microbial suppressiveness might have contributed to minimise *Phytophthora* disease incidence in SCM and HCM plots. Pepper fruit yield increased with manure amendment in SCM and HCM, which averaged 4.6 and 4.3 kg m^{-2}, respectively. Further research will be necessary to guarantee an effective *Phytophthora* biodisinfestation by fitting manure N and organic matter applications, improving crop yield and reducing greenhouse gas pollution.

1. Introduction

Phytophthora root rot, caused by the oomycete *Phytophthora capsici* Leonian, is a destructive disease for pepper plants (*Capsicum annuum* L.) worldwide. The mortality of pepper plants ranges between 30% and 40%, which when severe could be even up to 100% (Liu et al., 2008). As a consequence of the high plant mortality, relevant economic losses have been reported in pepper crops from Spain (Tello and Lacasa, 1997; Larregla, 2003). Strategies recommended for management of *Phytophthora* root rot involve integrated approaches that focus on cultural practices: reduced soil moisture, reduction of pathogen propagule in soil, utilisation of cultivars with resistance to the disease and the judicious fungicide applications (Ristaino and Johnston, 1999). In relation to the use of chemical fumigants, methyl bromide has been used for many years to provide successful season-long control of fungal, bacterial and nematode diseases. However, due to the environmental hazards associated to methyl bromide as an atmospheric ozone-depleting agent, it was practically removed from the market in 2005 (Gilreath and Santos, 2004). In this sense, the search for alternatives to methyl bromide had already begun several years earlier with the creation of the Methyl Bromide Technical Options Committee in 1992.

Soil solarisation and the application of organic amendments on soil have been described as a valid alternative to the use of chemical fumigants to reduce *Phytophthora* from pepper crops (Ristaino and Johnston, 1999). Soil solarisation is an approach to soil disinfestation which uses passive solar heating of soil with plastic sheeting, usually transparent polyethylene (Stapleton, 2000). The resulting soil temperature increase leads to decreased populations of pathogens. Combining soil solarisation with the amendment of fresh

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organic residues elevate soil temperature by an additional 1–3 °C, in addition to the generation of toxic volatile compounds which enhance the vulnerability of soil pathogens (Gamliel et al., 2000). This approach of combining soil solarisation together with the application of organic matter has been defined as biosolarisation (Ros et al., 2008) or biodisinfestation (de la Fuente et al., 2009). Organic amendments used for pathogen control are extremely heterogeneous, including green and animal manure together with agro-industrial by-products (Piedra Buena et al., 2007). Although less extended than green manure, the use of animal manure as a biofumigant agent is a remarkable alternative to reduce soil pathogens. The application of animal manure leads to the generation of ammonia (NH_3), which is the mechanism most often implicated in killing soil pathogens (Tenuta and Lazarovits, 2002), although other lethal molecules such as nitrous acid and volatile fatty acids (VFA) have also been reported (Tenuta et al., 2002; Conn et al., 2005). Tenuta and Lazarovits (2002) summarised that NH_3 is thought to kill cells by disrupting membranes, eliminating proton gradients across membranes, through the assimilation of NH_3 into glutamine and the exhaustion of the chemical energy of cells removing cytosolic NH_3. Other mechanisms involved in the control of soil pathogens by organic amendments may be the changes in soil pH and electric conductivity, the competition for nutrients as a consequence of the enhanced soil microbial activity (Leoni and Ghini, 2006) and the presence of antagonistic microorganisms in wastes (Pascual et al., 2009).

Despite the positive effect of NH_3 on soilborne pathogen control, NH_3 volatilisation from animal manure may also contribute to environmental damages such as acidification (Sommer and Hutchings, 2001). In addition to NH_3 emissions, the application of animal manure may be related to increasing emissions of nitrous oxide (N_2O) and carbon dioxide (CO_2) gases (Rochette et al., 2004). However, few data are available on the contribution of animal manure amendments on pollutant gases when used as biofumigants in crop production. The awareness on climate change and the increasing international policies such as the Gothenburg Protocol for NH_3 and Kyoto Protocol for Greenhouse Gases (GHGs) make interesting to study the effect of soil pathogen biodisinfestation by animal manure on NH_3 and GHG emission from crop production.

The objective of this experiment was to test the contribution of soil biodisinfestation by animal manure to NH_3 concentration and GHG emission (N_2O and CO_2). *Phytophthora capsici* disease rate and crop productivity after manure amendment were integrated in the study.

2. Materials and methods

2.1. Experimental design

In spring 2008, a greenhouse experiment was carried out at NEIKER-Tecnalia Research Station (Derio, 43°18′20″ N–3°53′0″ W). The region has a temperate climate with annual mean temperature of 12 °C (maximum mean temperature in summer 25 °C) and rainfall of 1200 mm yr^{-1}. Four treatments were replicated in three fully randomised blocks (28.2 m^2 per plot): control soil (C), solarisation (S), biodisinfestation through the combination of solarisation and the application of a mixture of fresh sheep manure and dry chicken litter (SCM) and biodisinfestation by solarisation and the amendment of a commercial semi-composted mixture of horse manure and chicken litter (HCM) applied at 1:1 ratio of volume. Table 1 shows the physical and chemical characteristics of SCM and HCM. Manure application was carried out at the same dry matter (DM) rate in both treatments, with 5.1 kg DM m^{-2} for SCM and HCM manure, respectively. Manure was incorporated into the soil (depth, 20 cm) using a rotavator on 12th March 2008. On 13th March, pepper (*Capsicum annuum* L., cultivar Derio) plant residues (basal stems and roots) infested with *P. capsici* (mycelia, sporangia, zoospores and oospores) were buried into the soil and therefore experimental plots were artificially infested. Soil was irrigated to field capacity with 27 L m^{-2} by a spray irrigation system. On 14th March the plots corresponding to S, SCM and HCM treatments were covered with a transparent polyethylene film (PE 200 gauge, 0.05 mm thick). Soil solarisation and biodisinfestation processes finished on 22nd April. After removing plastic sheets, pepper plants were planted on 25th April at a crop density of 33,670 plants ha^{-1}. A drip irrigation system was installed to keep accurate water status of plants, which were harvested on 22nd August.

Table 1
Physical and chemical characterisation of fresh sheep manure and dry chicken litter (SCM) and semi-composted mixture of horse manure and chicken litter (HCM).

	DM (%)[c]	pH	OM (%)[d]	N (%)	NH_4^+-N (%)[e]	NO_3^--N (%)[f]	N_{org} (%)[g]	C/N
SCM	43.4[a]	9.1[a]	45.4[a]	3.2[a]	0.37[a]	0.06[a]	2.77[a]	8.1[b]
HCM	42.5[a]	9.1[a]	45.4[a]	1.7[b]	0.29[a]	0.06[a]	1.35[b]	15.3[a]

[a,b]Columns followed by different upper case are different at significance level $P < 0.05$.
[c] DM, dry matter.
[d] OM, organic matter.
[e] NH_4^+-N, ammonium-N.
[f] NO_3^--N, nitrate-N.
[g] N_{org}, organic N.

2.2. Gas measurements

2.2.1. Ammonia

Ammonia was measured under polyethylene plastic sheets from 17th March to 21st April 2008. Measurements were recorded with a photoacoustic infrared gas analyser (Brüel and Kjaer 1302 Multi-Gas Monitor, detection limit 0.2 ppm for NH_3). The analyser was allowed to suck air under the plastic through connecting fittings placed on the plastic film. Gas sampling was conducted once a week at 10:00. As preliminary tests showed that NH_3 concentration was similar between C and S plots, information was obtained from measuring NH_3 in C treatment. Ambient and soil temperatures (15 cm depth) were continuously monitored in one S, SCM and HCM plot throughout the experiment using sensors connected to data-loggers (HOBO WEATHER STATION, Onset Computer Corporation, USA).

2.2.2. Nitrous oxide and carbon dioxide

Nitrous oxide and CO_2 emissions were measured from 22nd April to 3rd June 2008 once the plastic sheets had been removed. Measurements were recorded through a closed air circulation technique in conjunction with the photoacoustic infrared gas analyser (detection limit 0.03 ppm for N_2O and 3.4 ppm for CO_2). Emissions were assessed 2–3 times a week using PVC chambers (volume 6.75 L, area 0.0314 m^2), which were fitted tightly on to a plastic frame. Three frames were inserted 3 cm into the soil and repositioned to account for the spatial variation in each plot. Measurements were carried out for 40 min after the insertion of the chamber (Merino et al., 2001) and fluxes were calculated from concentration increase in the chamber headspace with time ($R^2 > 0.90$). Sampling was conducted daily from 10:00 to 14:00. Ambient and soil temperatures (10 cm depth) were monitored every sampling day using a portable thermocouple thermometer (HI 935009, Hanna instruments, Woonsocket, USA). As N_2O and CO_2 emissions were similar in C and S plots during the first measurements, these gases were measured only in C plots in the following determinations.