NITROUS OXIDE EMISSIONS FOLLOWING SOIL BIODISINFESTATION WITH ANIMAL MANURE ON A GREENHOUSE PEPPER CROP

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1 INTRODUCTION

Phytophthora blight, caused by oomycete *Phytophthora capsici*, is a devastating disease on pepper crops. Methyl bromide has been used for many years to provide control of the disease. However, it was removed from markets in 2005 due to environmental hazards. Solarisation through polyethylene sheets and soil biodisinfestation, which involves manure application under the plastic sheets, have been described as alternative processes to reduce disease incidence (Ristaino and Johnston, 1999). Biodisinfestation elevates soil temperature together with the generation of toxic volatile compounds, which enhance the vulnerability of pathogens (Piedra Buena et al., 2007). Among other lethal molecules, the application of animal manure leads to ammonia (NH_3) generation, which is the mechanism most often implicated in killing pathogens (Tenuta and Lazarovits, 2002). In contrast, the application of manure may be related to nitrous oxide (N_2O) emissions. Few data are available on the contribution of animal manure on N_2O emission when used as biofumigant. The objective of this trial was to test the contribution of soil biodisinfestation technique to NH_3 concentration and N_2O emission. Disease rate and crop yield were also analysed.

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). Four treatments were replicated in three fully randomised blocks (28 m^2): control soil (C), solarisation (S), biodisinfestation with a mixture of fresh sheep manure and dry chicken litter (SCM) and biodisinfestation with a commercial semicomposted mixture of horse manure and chicken litter (HCM) applied at 1:1 ratio of volume. Manure was incorporated into the soil (depth, 20 cm) using a rotavator on 12th March 2008. On 13th March pepper (*Capsicum Annuum* L., var Derio) plant residues infested with *Phytophthora capsici* were buried into soil. Plots were covered with a transparent polyethylene film (PE 200 gauge) on 14th March. 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} and harvested on 22nd August.

2.2 Gas measurements

Ammonia was measured under polyethylene sheets (connecting fittings placed on the film) from 17th March to 21st April 2008. Measurements were recorded with a photoacoustic infrared gas analyser (Brüel and Kjaer 1302 Multi-Gas Monitor). Sampling was conducted once per week and determinations began at 10:00 a.m.

Nitrous oxide 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 (PVC chambers volume 6.75 L, area 0.0314 m^2) in conjunction with the photoacoustic infrared gas analyser. Emissions were assessed twice or three times a week. 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 minutes after closing 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 always conducted in the same moment of the day (from 10:00 to 14:00). As N_2O emissions were similar in C and S plots during the first measurements, only C plots were measured for N_2O. Cumulative N_2O emissions were estimated by averaging the rate of loss between two successive determinations, multiplying the average rate by the length of the period between measurements, and adding that amount to the previous cumulative total.

2.3 Soil analysis

Soils, classified as clay soils (47.4% clay, 36.2% silt and 16.4% sand in the top 10 cm), were sampled (0-10 cm depth) and analysed before manure application for initial characterisation and weekly after removing plastic sheets. They were analysed for water-filled pore space (WFPS), pH, N (Kjeldahl-N method) and C:N ratio. Besides, ammonium and nitrate N content (NH_4^+–N and NO_3^-–N) were measured by segmented flow analysis. Potential N mineralisation (PNM) was calculated at the end of the experimental period. Soil temperature was continuously monitored at 15 cm.

2.4 Inoculum survival, disease incidence and crop yield

After the removal of the plastic films on 22nd April, 12 plant residues from each plot were collected and pathogen survival determined. The presence of *Phytophthora capsici* was confirmed using immature carnation petals as "vegetable traps". The incidence of disease was quantified in all treatments on a weekly basis until crop harvest. Plants were rated as "diseased" if shrivelled and crown rot lesions were observed. Plants were harvested on 22nd August and yields determined.

2.5 Statistical analysis

Data were analysed using SAS 8.0 Software. Prior to statistical analysis, emissions of N_2O were log-transformed. Data on disease incidence were transformed using arcsin ($\sqrt{x/n}$), where x= number of diseased plants and n= number of pepper plants in each experimental unit. Differences among treatments (cumulative gas emissions, inoculum survival rate, disease incidence and crop yield) were determined by analysis of variance (ANOVA) and means were separated by least significant difference (LSD) test. The LSD-test was also used for multiple comparisons of the instantaneous flux means. The model included manure as fixed factor, plots as random factor, and the interaction between treatments. Significance for ANOVAs as well as LSD-tests was determined at $P < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Manure application

Manure application was carried out at the same dry matter (DM) rate in both treatments, with 5.1 kg m^{-2} for SCM and HCM manure, respectively. Nitrogen content differed between manures ($P < 0.05$) and application doses were 0.16 and 0.09 kg N m^{-2} for SCM and HCM, respectively. These N loads were extremely high regarding the advised rates for green pepper fertilisation in the region (170 kg N ha^{-1}) (CBPA, 1999). As mineral N content did not differ in both manures ($P > 0.05$), the difference on N application was due to the different N organic content (N_{org}) of manure (2.77% DM in SCM and 1.35% DM in HCM).

3.2 Ammona concentration under plastic sheets

As Figure 1 shows, NH_3 concentration increased significantly after manure amendment with respect to C plots and differed between SCM and HCM manure ($P < 0.05$). Mean concentrations were 3.9 mg NH_3 m^{-3} for C plots, 14.8 mg NH_3 m^{-3} for SCM and 9.1 mg NH_3 m^{-3} for HCM. Ammonia concentration decreased 45.0% in manure amended treatments after 35 days of biodisinfestation. This trend might be related to the high water condensation observed on the inner surface of plastics, which might have trapped volatilised NH_3. Ammonia emission is regulated by NH_4^+-N concentration of the solution and is modelled by factors such as pH or temperature. As pH-value did not differ in sampled soils (mean soil pH, 6.9) and manure (mean manure pH, 9.1), and soil and air temperatures were similar for all treatments, the difference on NH_3 concentration might be attributed to the different NH_4^+-N content. The higher N_{org} content in SCM, together with the lower C/N ratio (8.1 in SCM and 15.3 in HCM) which would have favoured N_{org} mineralisation (soil PNM was 197.7 and 88.9 mg NH_4^+-N kg^{-1} in SCM and HCM, respectively), might have contributed to higher NH_4^+-N availability in SCM plots.

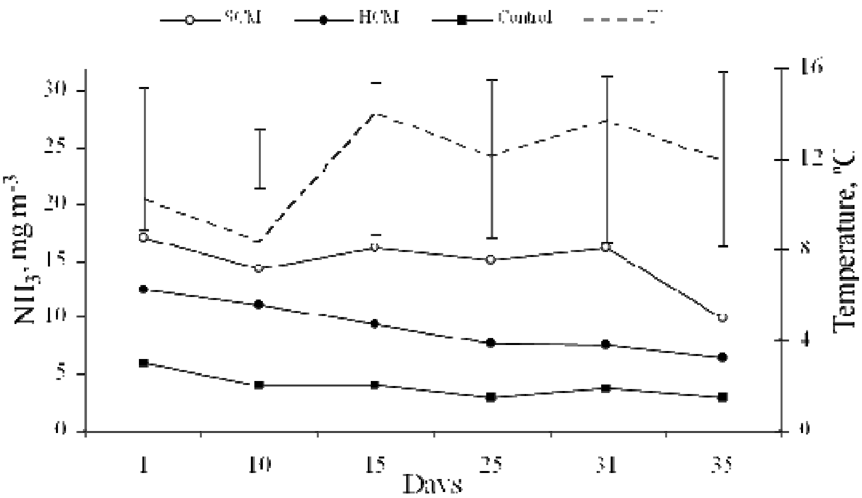

FIGURE1 **Ammonia concentration under plastic sheets and soil temperature.**

3.3 Nitrous oxide emission after biodisinfestation process

Nitrous oxide averaged 0.1 mg N_2O-N m^{-2} d^{-1} from C plots and amendment of both manure increased N_2O emissions ($P < 0.05$). The emission pattern was similar for SCM and HCM during the experiment: the highest peaks were observed for the first day, emissions remained stable for 2 weeks and decreased from then on (Figure 2).

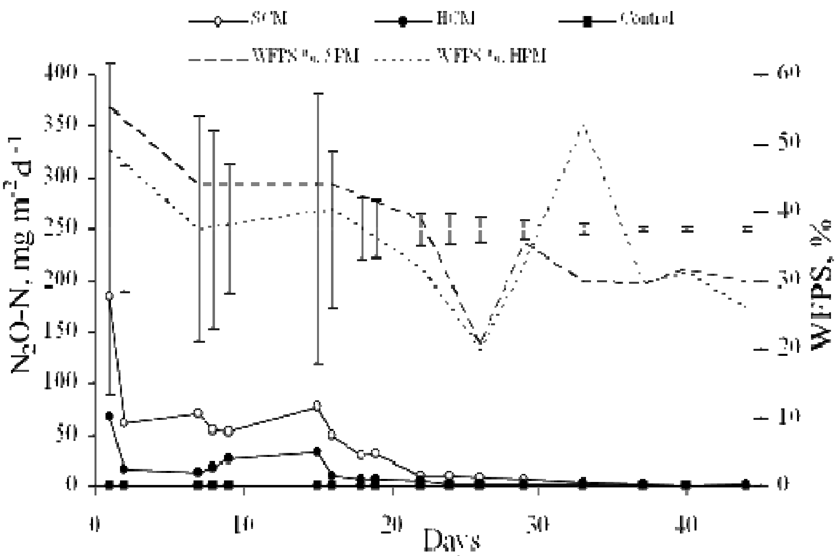

FIGURE2 **Nitrous oxide emission after biodisinfestation process and soil WFPS.**

Initial high peaks were related to N_2O cumulated under plastic sheets during soil biodisinfestation. We suggest that denitrification might have leaded to initial N_2O emission peaks. Soil nitrate content (9.4 g NO_3^--N m^{-2}), the water condensation and anaerobic conditions under the plastic sheets would have favoured conditions for denitrification. However, the evolution of soil mineral N showed a net nitrification process after removing plastic sheets. Soil NH_4^+-N content decreased for the first week, reducing by 92.8% and 83.7% the initial concentration in SCM (47.5 g NH_4^+-N m^{-2}) and HCM (13.8 g NH_4^+-N m^{-2}). In contrast, initial soil NO_3^--N content in SCM (13.8 g NO_3^--N m^{-2}) and HCM (6.1 g NO_3^--N m^{-2}) increased through 35 days of measurements. Nitrification would have been favoured by dry soil moisture conditions (Firestone and Davidson, 1989) and soil aireation. Cumulative N_2O emissions from SCM and HCM increased in relation to C treatment (0.01 g N_2O-N m^{-2}) ($P < 0.05$), but did not differ significantly between them (1.31 and 0.42 g N_2O-N m^{-2} in SCM and HCM, respectively). As N application was higher from SCM manure, N_2O emission factor was calculated to compare both treatments. Data showed that N_2O losses represented 0.80% and 0.47% of applied N in SCM and HCM manure, respectively. This suggested that the application of fresh manure might contribute to larger N_2O emissions due to lower N_{org} stability.

3.4 Inoculum survival, disease incidence and crop yield

The application of fresh SCM under plastic sheets reduced *Phytophthora* inoculum survival (30.6%) in relation to HCM (75.0%) and S treatments (94.4%) ($P < 0.05$). We hypothesize that higher inactivation observed in SCM could be attributed to the effect of toxic volatile compounds, including higher NH_3 concentration. In addition, as temperature did not exceed 33ºC, solarisation did not succeed in reducing inoculum survival rate. *Phytophthora* disease incidence differed among treatments after 4 months since crop transplanting ($P < 0.05$). Manure amended treatments reduced significantly the incidence (2.8% for SCM and 8.3% for HCM) compared with C and S (40.7% and 42.6%, respectively). Plant disease incidence was reduced by 90% in HCM, despite the high inoculum survival rate observed. This phenomenon could be explained through the increment in soil suppressiveness, in which soil microbes play an important role by an antagonistic mechanism (Hoitink and Boehm, 1999). Pepper crop yield differed among treatments ($P < 0.05$). Production increased with manure amendment, averaging 4.6 and 4.3 kg m^{-2} in SCM and HCM, respectively. When crop yield was related to cumulated N_2O emission, results showed that SCM averaged 0.28 g N_2O-N kg^{-1} pepper while HCM averaged 0.10 g N_2O-N kg^{-1} pepper. These results were accounted for the high N inputs incorporated from manure.

4 CONCLUSIONS

The use of fresh manure might favour NH_3 volatilisation, as N_{org} mineralisation rate might be higher than in semicomposted manure. However, animal manure application increased N_2O emission. Larger N_{org} mineralisation rate on fresh manure amended soils might have contributed to higher N_2O emissions during and after soil biodisinfestation by denitrification and nitrification processes, respectively. The application of animal manure followed by plastic covering during spring period reduces the incidence of *Phytophthora capsici* in pepper crops. Ammonia volatilisation, among other volatile compounds, might contribute to minimize inoculum survival rate and disease incidence after biodisinfestation. Soil solarisation was not a successful strategy reducing pathogen incidence. Further research will be necessary to guarantee an effective *Phytophthora capsici* biofumigation by fitting manure N and organic matter applications, getting an efficient crop yield and reducing N_2O pollution.

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