

Effects of a nitrification inhibitor on the metabolic activity of ammonia oxidisers

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Introduction

Utilization of nitrification inhibitors (NI) for ammonium-based fertilizers is a powerful tool to reduce N_2O emissions and nitrate leaching. 3,4-Dimethylpyrazol-phosphate (DMPP) is a popular NI, which can delay the first and rate-limiting step of nitrification, ammonia oxidation and subsequent denitrification. But its efficiency at reducing N loss is highly variable across studies, as is the case for all of the NIs. It is normally accepted that ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) encoding the *amoA* gene catalyses ammonia oxidation. Several recent studies have reported that DMPP could decrease the abundance and change the community structure of the ammonia oxidisers. However, no study has revealed how DMPP addition would affect the metabolic activity of the ammonia oxidisers and is hypothesised to be more direct evidence for the effect of DMPP. This paper describes an experiment designed to identify the changes in the metabolic activity of ammonia oxidisers in two Australian dryland soils using DNA-stable isotope labelling method.

Materials and methods

- Two Australian upland soils, one from the vegetable site at Clyde (38°07' S, 145°19' E) and the other from the pasture at Dookie (36°25' S, 145°42' E), were collected and sieved (≤ 2 mm) for microcosm incubation with 60% water-filled pore space (WFPS) at 25 °C. Soil properties are shown in Table 1.
- Three treatments were set up: (1) NH_4NO_3 + 5% (v/v) $^{12}CO_2$, (2) NH_4NO_3 + 5% (v/v) $^{13}CO_2$, (3) NH_4NO_3 + 5% (v/v) $^{13}CO_2$ plus DMPP (1% of applied NH_4^+-N). Nitrogen was provided at 75 mg NH_4^+-N kg $^{-1}$ soil and 75 mg NO_3^--N kg $^{-1}$ soil.
- DNA was extracted from 0.25 g of each soil sample and subsequently used for gradient fractionation and quantitative real-time PCR.
- Ammonium and nitrate was extracted by shaking with 1M KCl (1:5) and measured with continuous flow colourimetric analyzer.

Results

- ^{13}C - CO_2 or DMPP showed no effect on the concentrations of NH_4^+-N and NO_3^--N in the pasture soil (Fig. 1A, C). While DMPP addition significantly slowed down the decline of the NH_4^+-N concentrations and reduced the increase of the NO_3^--N concentrations in the vegetable soil (Fig. 1B, D).
- DMPP significantly decreased ($P < 0.05$) the AOB abundance by 54.9% in the pasture soil and 50.2% in the vegetable soil on day 28, but showed no impact on AOA in the two soils (Fig. 2).
- The majority of the AOB communities were detected in the 'heavy' SIP fractions with a buoyant density of 1.73-1.76 g mL $^{-1}$ in the ^{13}C - CO_2 treatments compared with ^{12}C - CO_2 (1.72-1.73 g mL $^{-1}$) treatments on day 28 (Fig. 3E, F).
- DMPP shifted the peak of AOB towards the 'light' fractions in the vegetable soil on day 28, while had no obvious inhibition in the pasture soil (Fig. 3). DMPP had no obvious inhibitory effect on the metabolic activity of AOA in both soils (data not shown).

Table 1. Soil key properties

| Soil | Clay | Silt | Sand | SOM | pH | NO_3^--N | NH_4^+-N | Total N | CEC |
|-----------|------|------|------|------|-----|---------------|---------------|---------|-----------------|
| | (%) | (%) | (%) | (%) | | mg kg $^{-1}$ | mg kg $^{-1}$ | (%) | cmol kg $^{-1}$ |
| Pasture | 11 | 30 | 59 | 5.96 | 5.6 | 26.59 | 9.14 | 0.38 | 10.2 |
| Vegetable | 4 | 11 | 85 | 3.11 | 7.2 | 53.89 | 9.30 | 0.28 | 12.7 |

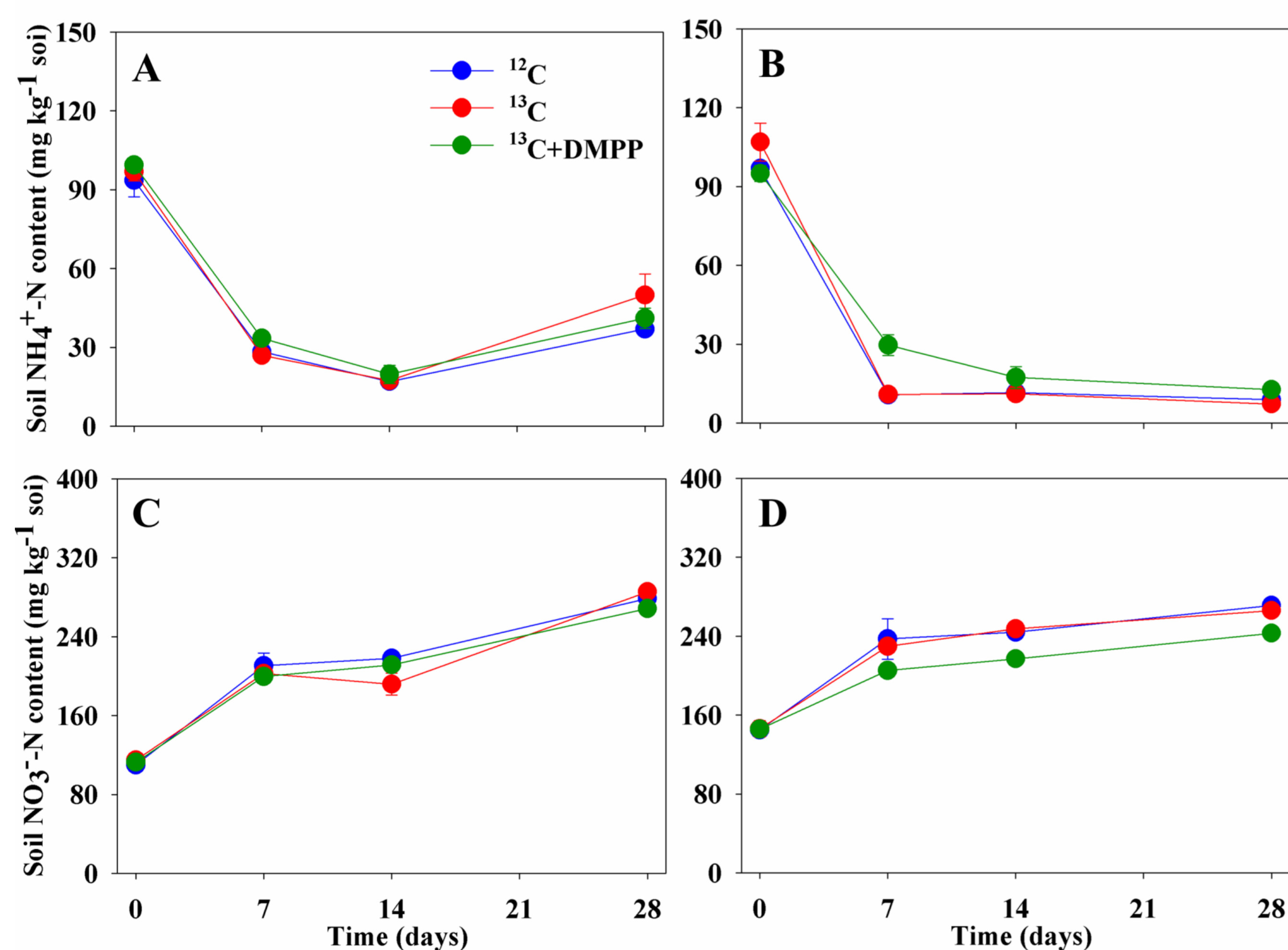


Fig. 1 Changes in the NH_4^+-N and NO_3^--N content in the pasture soil (A, C) and vegetable soil (B, D). Error bars represent standard errors of three replicates.

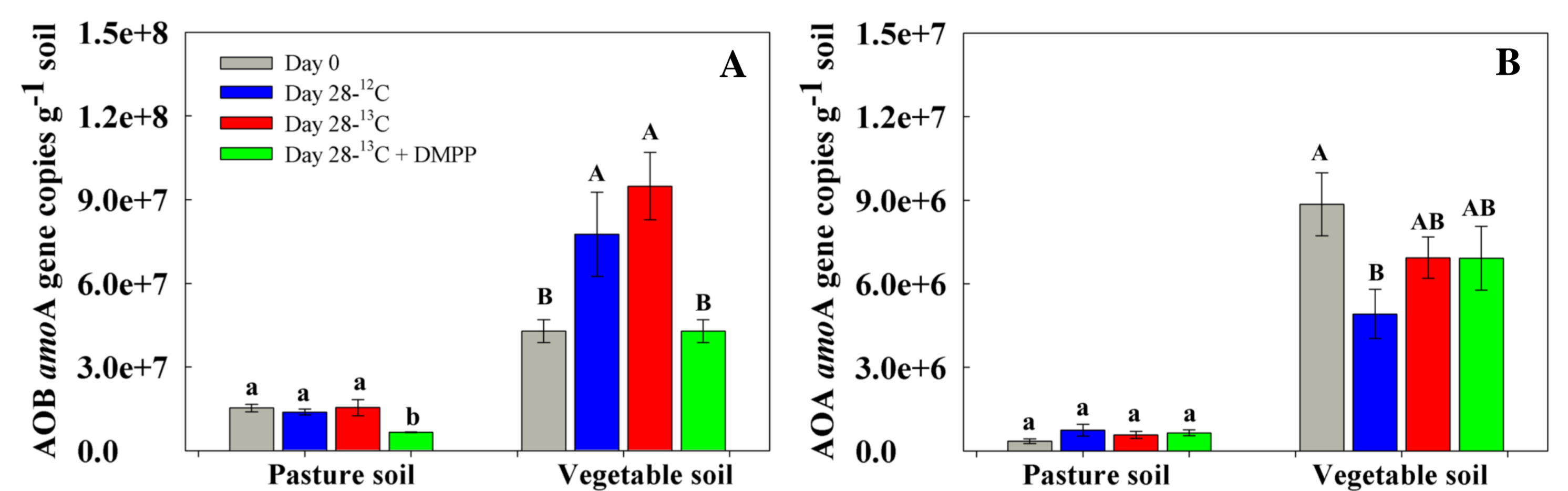


Fig.2 Changes in the AOB (A) and AOA (B) *amoA* gene copies across treatments in the DNA-SIP microcosms of the pasture soil and vegetable soil. Different letters above the bars indicate significant differences ($P < 0.05$) among treatments within the same soil.

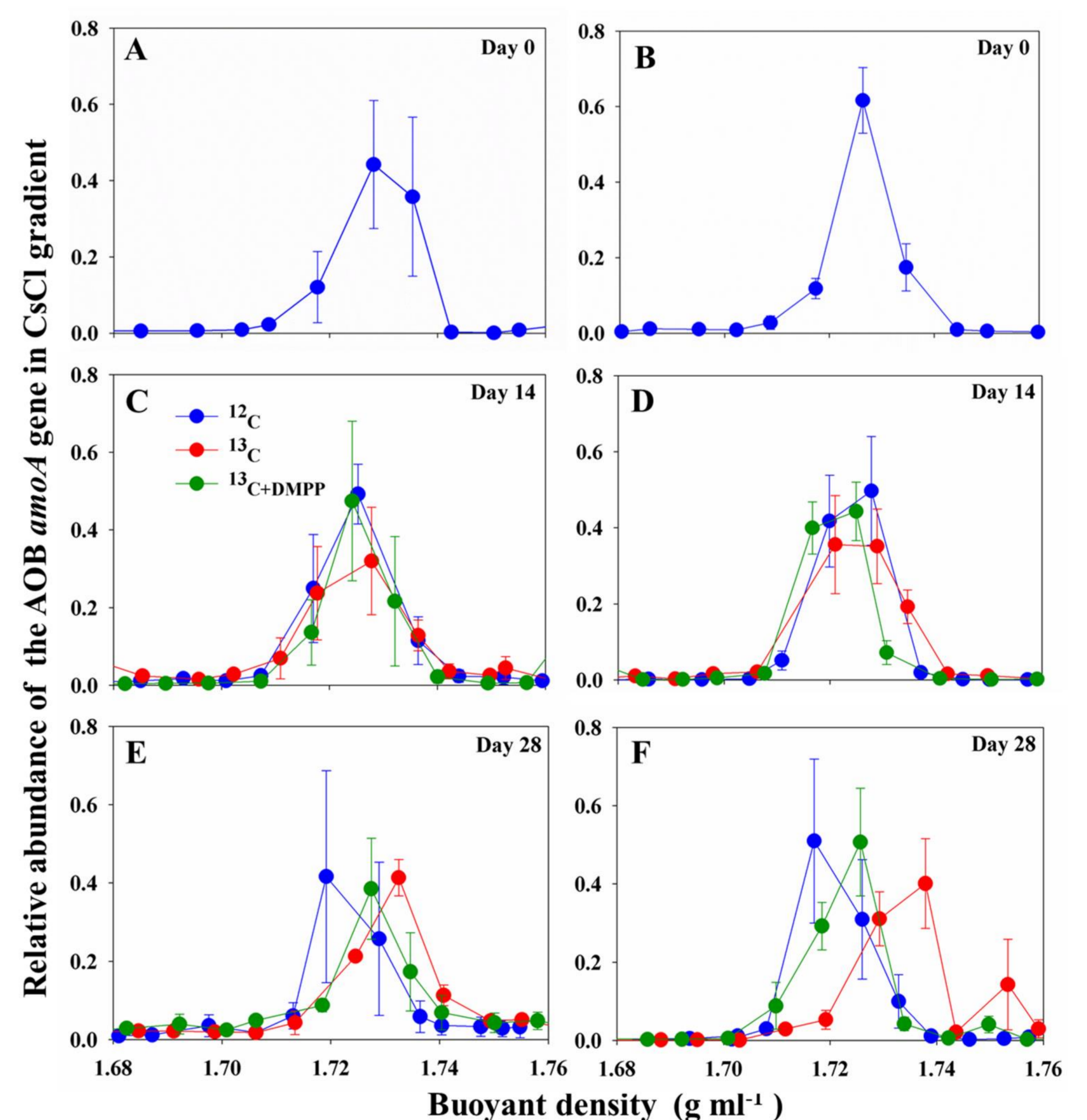


Fig.3 Relative abundances of the AOB *amoA* genes retrieved from different treatments in DNA-SIP microcosms of the pasture soil (A, C, E) and vegetable soil (B, D, F) on days 0, 14 and 28. Error bars represent standard errors of three replicates.

Conclusion

- DMPP was much more efficient in maintaining higher NH_4^+-N and slowing down NO_3^--N formation in the neutral vegetable soil compared with the acidic pasture soil.
- DMPP effectively inhibited nitrification through decreasing the abundance and metabolic activity of AOB (the assimilation of labelled CO_2 into the *amoA* gene) in the vegetable soil, but not in the pasture soil.

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