Title: Biocrusts modulate responses of nitrous oxide and methane soil fluxes to simulated climate change in a Mediterranean dryland

Running title: Biocrusts, climate change and greenhouse gas fluxes

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Abstract

Little is known about the role of biocrusts in regulating the responses of N_2O and CH_4 fluxes to climate change in drylands. Here, we aim to help filling this knowledge gap by using an eight-year field experiment in central Spain where temperature and rainfall are being manipulated (~1.9 °C warming, 33% rainfall reduction, and their combination) in areas with and without well-developed biocrust communities. Areas with initial high cover of well-developed biocrusts showed lower N_2O emissions, enhanced CH_4 uptake and higher abundances of functional genes linked to N_2O and CH_4 fluxes compared with areas with poorly-developed biocrusts. Moreover, biocrusts modulated the responses of gases emissions and related functional genes to warming and rainfall reductions. Specifically, we found under rainfall exclusion and its combination with warming a sharp reduction in N_2O fluxes (~96% and ~197%, respectively) only under well-developed biocrust cover. Warming and its combination with rainfall exclusion reduced CH_4 consumption in areas with initial low cover of well-developed biocrust, whereas rainfall exclusion enhanced CH_4 uptake only in areas with high initial cover of well-developed biocrusts. Similarly, the combination of warming and rainfall exclusion increased the

abundance of the *nosZ* gene compared to the rainfall exclusion treatment and increased the abundance of the *pmoA* gene compared to the control, but only in areas with low biocrust cover. Taken together, our results indicate that well-developed biocrust communities could counteract the impact of warming and altered rainfall patterns on soil N_2O and CH_4 fluxes, highlighting their importance and the need to preserve them to minimize climate change impacts on drylands.

Keywords

Biocrust, *denitrifiers*, dryland, methane, *methanotrophs*, nitrous oxide.

Highlights

- Under the combination of rainfall exclusion and warming, biocrusts reduced N_2O fluxes.
- Biocrusts enhanced the rate of CH_4 uptake.
- Soils with high biocrust cover had higher abundances of *pmoA* and *nosZ* genes.

Introduction

Most efforts to understand the main drivers of soil greenhouse gas (GHG) fluxes under global change scenarios have focused on carbon dioxide (CO_2) (Pachauri and Meyer 2014). Much less is known about other greenhouse gases such as nitrous oxide (N_2O), and methane (CH_4), which have stronger greenhouse effects and can significantly affect feedback responses to climate change (Nakicenovic and Swart 2000; Le Mer and Roger 2001; Soussana and others 2007; Oertel and others 2016). This is especially true for dryland (arid, semi-arid and dry-subhumid) ecosystems, which cover ~45% of the land surface (Prăvălie 2016) and sustain over 40% of human population (Reynolds and others 2007). The exchange of N_2O and CH_4 between the soil and the atmosphere has been traditionally considered of little importance in drylands due to their typically low water and nutrient contents, which limit biological activity (Dalal and Allen 2008). However, over the last two decades multiple studies have reported elevated N_2O fluxes in dryland soils after rainfall pulses (Barton and others 2008, 2013; Zaady and others 2013), and have noted their potential as a relevant global sink of atmospheric CH_4 (Potter and others 1996; Angel and Conrad 2009). Furthermore, the relevance of drylands as a contributor to the global balance of GHG fluxes will increase in the future, as their global extent will likely increase by 11-23% by the end of this century due to climate change (Huang and others 2016). However, how warming and forecasted changes in rainfall patterns will affect N_2O and CH_4 fluxes in drylands remains poorly studied (Darrouzet-Nardi and others 2015; Guan and others 2019).

Soil N_2O and CH_4 transformations are largely carried out by highly specialized microbial communities. For instance, the N_2O produced in both nitrification and denitrification processes (Firestone and Davidson 1989; Bremner 1997; Canfield and others 2010) is reduced to N_2 by the *nosZ* carrying denitrifiers under anaerobic conditions (Bremner 1997; Canfield and others 2010). In dryland

surface soils, aerobic nitrification has been traditionally considered the dominant process (Delgado-Baquerizo and others 2016). Consequently, the *nosZ* gene (carried by denitrifying bacteria) and the factors affecting its abundance and activity have been poorly studied (Philippot and others 2007; Hallin and others 2018). However, aggregates and precipitation pulses create anaerobic conditions favourable for denitrification in dryland soils, which could represent a temporary sink for atmospheric N_2O, the substrate used by *nosZ* denitrifiers (Austin and others 2004; Ley and others 2018; Wang and others 2019). Likewise, under aerobic conditions (dominant in dryland soils), CH_4 oxidizing bacteria use the CH_4 monooxygenase (encoded by the *pmoA* gene) to oxidize CH_4 , constituting the only biological sink for atmospheric CH_4 (Dalal and Allen 2008; Conrad 2009). Previous experiments and observational studies (Nazaries and others 2013; Powell and others 2015) have shown strong relationships between the abundance of *nosZ*/*pmoA* genes and GHG fluxes, and consequently functional genes have been used to predict these fluxes (Nazaries and others 2013; Powell and others 2015). Unfortunately, most of our knowledge on *nosZ* and *pmoA* genes comes from mesic ecosystems (Nazaries and others 2013; Powell and others 2015; Martins and others 2017; but see Martins and others 2015, Lafuente and others 2019), and we lack studies evaluating the changes in their abundance under global change scenarios in drylands.

Biocrusts, soil surface communities composed by lichens, mosses, liverworts, fungi, algae, cyanobacteria and other microorganisms, are a key biological component of dryland ecosystems worldwide (Weber and others 2016). Biocrusts regulate a myriad of key soil biotic and abiotic properties and processes (Eldridge and others 2010; Aschenbach and others 2013; Maestre and others 2013; Zaady and others 2013; Felde and others 2014), and are home to particular soil microbial communities (Steven and others 2013; Delgado-Baquerizo and others 2018). However, and to the best of our knowledge, no previous field studies have experimentally

evaluated how biocrusts influence soil N_2O and CH_4 fluxes under simulated climate change. Such studies are needed not only to advance our understanding of climate change impacts on drylands, where biocrusts are a prevalent biotic feature, but also to provide relevant data to refine simulation models employed to forecast future N_2O and CH_4 fluxes across dryland biomes.

Herein, we used an eight-year (2008-2016) warming and rainfall manipulation experiment located in central Spain (Maestre and others 2013) to investigate: (i) the effects of simulated climate change (~1.9 ºC warming and ~33% rainfall reduction) on soil N_2O and CH_4 fluxes and the abundance of *nosZ* and *pmoA* functional genes; (ii) whether these effects are modulated by biocrusts; and (iii) the relationships between N_2O and CH_4 fluxes and the abundance of *nosZ* and *pmoA* functional genes, respectively.

Materials and methods

Study site

This experiment was conducted in the Aranjuez Experimental Station (central Spain; 40º01'55.7''N-3º32'48.3''W; 590 m.a.s.l; for more details on this experimental station see http://maestrelab.blogspot.com/2013/05/the-aranjuez-experimental-station.html). Its climate is Mediterranean semi-arid, with average annual temperature and rainfall of 15°C and 358 mm, respectively (data available since 1977 from the Aranjuez Meteorological Station, 40º04'N - 3º32'W; 540 m.a.sl). Soils are gypsum-derived (Gypsiric Leptosols, WRB 2006). Organic carbon (C), total nitrogen (N), and pH vary among the considered microsites (i.e. areas with low and high biocrust cover) between 1.8-5.0%, 0.14-0.44%, and 6.6-7.2, respectively. Vegetation is dominated by *Macrochloa tenacissima* (L.) Kunth (18% of total cover), *Retama sphaerocarpa* (L) Boiss and *Helianthemun squamatum* Pers. (6% of total cover for both shrubs).

Open areas between vascular plants are partially covered with a well-developed biocrust community dominated by lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt, *Toninia sedifolia* (Scop.) Timdal, and *Psora decipiens* (Hedw.) Hoffm, which covers ~34 % of the soil surface (see Maestre and others 2013 for a full species checklist).

Experimental design

A detailed description of the experimental design can be found in Escolar and others 2012. Briefly, we established a fully factorial experimental design with three factors, each with two levels: warming (control vs. ~1.9 ºC soil temperature increase), rainfall exclusion (control vs. 33% rainfall reduction) and biocrust cover (<25% vs. >50% of lichens and mosses; hereafter low and high biocrust cover, respectively). To simulate warming, we used 40 × 50 × 32 cm hexagonal open top chambers (OTCs) made of methacrylate, which were elevated 5 cm from the surface to avoid overheating (Fig. S1a). To intercept rainfall, we built a 1.2 × 1.2 × 1 m metallic frame supporting three V ~37% of the surface (Figure S1b). Warming and rainfall exclusion treatments were setup in July and November 2008, respectively (see Maestre et al. 2013 for additional details). Ten replicates per combination of treatment were established, resulting in 80 experimental plots. Our warming treatments (warming and its combination with rainfall exclusion), significantly increased soil temperatures by ~1.6°C and 2.3°C, respectively, compared to control plots (average of the 2008-2019 period). Our rainfall exclusion (RE) shelters excluded on average ~33% of the incoming rainfall (2008-2013 period).

At each plot we inserted a polyvinyl chloride (PVC) ring (diameter = 20 cm, height = 7 cm) 5 cm into the ground at the start of the experiment for measuring GHG fluxes and monitoring changes in biocrust cover. Well-developed biocrust cover (i.e. lichens and mosses) was estimated annually using high resolution pictures since the setup of the experiment in 2008, as detailed in Maestre and others (2013). Values obtained using these pictures are highly correlated with those obtained with *in situ* surveys (Ladrón de Guevara and others 2018).

Soil moisture (0-5 cm) and temperature (0-2 cm) were monitored every 2.5 h and 0.5 h, respectively in all treatments in a subset of the plots using EC-5 soil moisture (Decagon Devices Inc., Pullman, WA, USA) and HOBO® TMC20 (Onset Corporation, Bourne, MA, USA; Figs. S2 and S3) sensors.

Greenhouse gas exchange measurements

We estimated soil-atmosphere N_2O and CH_4 fluxes in seven replicates per combination of treatments using the static chamber method (Bowden and others 1990). From March 2015 to May 2016, 14 sampling campaigns were carried out approximately once a month. Immediately before each measurement, a 20 cm diameter and 9 cm high PVC chamber was placed on top of each of the 56 permanent rings and sealed with a rubber band. Each chamber had a sampling port in the top centre that allowed air sampling and was covered with reflective material to thermally isolate it during the measurement. Gas samples were collected at 0, 30 and 60 min after chamber closure using a needle attached to a polypropylene syringe, transferred to 22 ml pre-vacuumed vials and kept at room temperature until analysis. We estimated N_2O and CH_4 concentrations in the gas samples using a HP-6890 gas chromatograph (GC), equipped with a

129 headspace autoanalyzer (HT3) (Agilent Technologies, Barcelona, Spain), a ^{63}Ni electron capture detector (for N_2O), and a flame-
130 ionization detector fitted with a methaniser (for CH_4 detection). The carrier gas used was helium.

131 *Soil sampling and analyses*

132 Soil samples (0-2 cm) were collected five times during the study period (June, September and November 2015 and February and April
133 2016) from five replicates per combination of treatments. Each soil sampling always matched one of the gas measurement campaigns.
134 Visible biocrusts (i.e. lichens and mosses) were removed when present and then soils were stored in -20 ºC for DNA extractions.

135 Total genomic DNA was extracted from 0.6 g of frozen soil using the PowerSoil DNA Isolation kit (MOBIO Laboratories, Inc.
136 USA) according to manufacturer's protocol but with a slight modification during the cell lysis step (we used a tissue homogenizer
137 [Precellys 24- dual. Bertin technologies, France] at a speed of 4500 rpm for 45 s, twice). DNA extractions yields ranged from 0.1 to
138 132.6 ng/µl, with an average of 6 ± 15 ng/µl (mean ± standard deviation, n = 5). The abundances of *nosZ* and *pmoA* genes were
139 determined using nosZ2f/nosZ2r (Henry and others 2006) and pmo189f/pmo650r (Bourne and others 2001) primers, respectively. All
140 primers were purchased from Integrated DNA Technologies (Australia). Each sample was quantified (in duplicate) in a total volume of
141 10 µl using a BioRad C1000 Touch thermal cycler CFX96 Real-Time System (Bio-Rad Laboratories, USA). The reaction mixture
142 contained 1 µl of DNA template (2 ng/µl; those samples with a concentration <2 ng/µl were not diluted in sterilised water), 5 µl of
143 SensiFast Sybr No-Rox Mix (2x) (Bioline, Australia), 0.3 µl of each primer (0.4 mM) and 0.4 µl of BSA (0.4 mg/ml). Thermal cycling
144 conditions and primer sequences can be found in Table S1. The *nosZ* gene was cloned with pGEM-T Easy Vector kit according to
145 manufacturer's instructions (Promega, Madison, USA) and transformed into *Escherichia coli* strain JM109 to perform calibration

curves. The *pmoA* gene calibration curves were made form genomic DNA (*Methylosinus trichosporium*). Melt curve analyses were performed in each assay to verify the specificity of the amplicon products. Gene copy number per g dry soil normalized to extraction yield were calculated for both genes.

Statistical analyses

We estimated N_2O and CH_4 fluxes as described in Durán and others (2013), and reported them as changes in milligrams or micrograms (for N_2O) per square meter per day. In more than 90% of the cases, the increases in N_2O and CH_4 emissions were linear (R^2 >0.7). Non-linear rates were discarded, and imputation of missing rates (per treatment) was performed using the missForest algorithm in the R package missForest (Stekhoven and Buehlmann 2012), which iteratively fills missing values in all columns of a data frame based on predictions from random forest models. For the iteration, we included the averaged soil moisture and temperature matching the treatment, date and time of the sampling. We estimated the 2.3% and 6.8% of the N_2O and CH_4 rates analysed in this study, respectively.

We first tested the effects of warming, rainfall exclusion and biocrust cover (i.e. low and high biocrust cover in the ring when the experiment was established in 2008) on N_2O and CH_4 fluxes and soil microbial gene abundance (*nosZ* and *pmoA* functional genes) with a repeated measures general linear mix effects model. We also included the rate of change in the biocrust cover over time (in %) as a covariate in the models to control for the observed changes in biocrust cover since the setup of the plots in 2008 (described in detail in Ladrón de Guevara and others 2018). As multiple interactions between initial biocrust cover and the climate change treatments were found (Table 1), we tested the effect of warming and rainfall exclusion (alone and combined) separately for low and

high biocrust cover plots using the same model. These analyses were carried out using the function *lmer* in the R package *lmer4* (Bates and others 2015). Differences of least squares means for the factors of the mixed effects model were calculated using the function *difflsmeans* in the R package *lmerTest* (Kuznetsova 2017) with no p-value adjustment. We compared differences in gas fluxes and gene abundances between the two levels of biocrust cover (low and high), with the student's t-test. Methane fluxes and soil microbial gene abundances were log transformed prior to analyses to improve normality. All statistical analyses were performed using R statistical software 3.4.0 (R Core Team 2017). Data are available on Figshare (Lafuente and others 2020).

Results

Effects of simulated climate change on N_2O and CH_4 fluxes

Nitrous oxide fluxes were very low in all cases, ranging on average from -10 to 20 μg m^{-2} d^{-1}, and had a high temporal variability (Figs. S4a,b, and 1a,b). These fluxes did not differ between biocrust cover levels (Fig. 1 a,b; p=0.14; Fig. S5a). Biocrusts, however, regulated the responses of N_2O emissions to warming and rainfall exclusion. In low biocrust cover plots, these climatic manipulations reduced N_2O fluxes (vs. control) in March, April and early July, and increased them in late July and September (Fig. S4a). However, in high cover plots we observed sharp reductions in N_2O fluxes in the rainfall exclusion and warming + rainfall exclusion treatments as compared with the control plots (~96% and ~197%, respectively; Fig. 1b, $p < 0.05$, Table S2).

Methane fluxes were also low and negative (i.e. CH_4 uptake) in most cases, and ranged on average from -1.66 to -1.22 mg m^{-2} d^{-1} (Figs. S4c,d and 1c,d). The CH_4 uptake was higher in high (vs. low) biocrust cover plots (p<0.01; Fig. S5b). The response of CH_4

fluxes to warming and rainfall exclusion was very variable throughout the study period, and was modulated by biocrust cover (Fig. 1c,d). All climate change treatments tended to decrease CH_4 uptake in low biocrust cover plots, although these differences were only found in the warming and warming + rainfall exclusion treatments (Table S2, Fig. 1c). However, in high biocrust cover plots, only warming reduced CH_4 uptake (Table S2, Fig. 1d).

Effects of climate manipulation on the abundances of nosZ *and* pmoA *genes*

Both *nosZ* and *pmoA* genes were more abundant in high than in low biocrust cover plots ($p<0.05$; Fig. S5c,d). As found with N_2O and CH_4 fluxes, we observed a marked variability in *nosZ* and *pmoA* gene abundance throughout the experiment (Fig. S6), as well as important differences in their responses to warming and rainfall exclusion treatments depending on biocrust cover (Figs. S6 and 2).

The averaged abundances of the *nosZ* gene ranged from $2.4x10^5$ to $7.8x10^7$ copy number g dry $soil^{-1}$ (Table S3, Fig. 2a, b). Warming, rainfall exclusion, and their combination reduced the abundance of the *nosZ* gene (vs. the control) in the September sampling. In low biocrust cover plots, its overall abundance was higher at the warming + rainfall exclusion treatment than at the rainfall exclusion treatment (Table S3, Fig. 2a). We did not find any relationship between N_2O fluxes and *nosZ* gene abundance (R^2 = 0.00; p=0.91 and R^2 = 0.02; p=0.07 in low and high biocrust cover plots, respectively).

On average, the *pmoA* gene abundance ranged from $1.6x10^4$ to $8.3x10^5$ copy number g dry $soil^{-1}$ (Fig. 2c,d). The combination of warming and rainfall exclusion led to an overall increase in the abundance of the *pmoA* gene, but only in the low biocrust cover plots (Fig. 2c,d; Table S3). A positive relation between CH_4 fluxes and the abundance of the *pmoA* gene was observed in the warming + rainfall exclusion plots, but only in low biocrust cover plots (R^2 = 0.13; p=0.04).

Discussion

Our study provides novel experimental evidence that biocrusts are key regulators of the responses of N_2O and CH_4 fluxes and associated functional genes to climate change drivers. Biocrusts regulated the temporal patterns of N_2O and CH_4 fluxes and their response to our climate change treatments. For instance, and despite being highly variable in space and time, the combination of warming and rainfall exclusion led to a sharp reduction (197%) in average N_2O fluxes, but only in areas with high biocrust cover. Biocrusts also mitigated reductions in CH_4 uptake observed under the combination of warming and rainfall exclusion. These results highlight the importance of considering biocrusts when assessing ecosystem responses to climate change in drylands and when estimating future GHG fluxes from soils in these ecosystems, which are forecasted to cover more than 50% of the terrestrial surface by the end of this century (Huang and others 2016).

Our results suggest that projected changes in temperature and precipitation will likely modify the capacity of dryland soils to exchange N_2O with the atmosphere. More importantly, these findings indicate that such responses depend on the degree of biocrust development. While in low biocrust cover areas rainfall exclusion (and its combination with warming) tended to increase N_2O emissions throughout the study period, this treatment promoted a sharp decrease in these emissions when well-developed biocrust communities were present. Furthermore, soils at high biocrust cover plots were a net N_2O sink under the combination of warming and rainfall exclusion. These results highlight the ability of biocrusts to mitigate the effects of climate change on N_2O emissions, but also the importance of considering the interactions among different climate change drivers when evaluating potential future GHG emissions

(Fig. S4b). Interestingly, and despite the reductions in biocrust cover induced by warming over the years in our experiment (Ladrón de Guevara and others 2018), we still found a sharp reduction in N_2O fluxes in the rainfall exclusion and its combination with warming treatments. Such a result suggests a strong legacy effect of biocrusts on soil functioning, similar to that reported in other mesic ecosystems with plants (Meisner and others 2013), and further highlights the importance of these communities on driving the responses of drylands to climate change drivers.

Climate change effects on N_2O fluxes are highly variable due to the key importance of climatic factors such as soil temperature and moisture as drivers of GHG emissions (Dijkstra and others 2012; Zhou and others 2016). Warming, and the associated increases in soil temperature, could enhance the metabolism of nitrifiers and denitrifiers, boosting N_2O emissions (Dalal and others 2003) (Fig. S3). However, and particularly in drylands, climate change-driven reductions in soil moisture (either associated with warming or due to decreases in precipitation) can limit microbial metabolism and thus reduce atmospheric N_2O emissions (Chapuis-Lardy and others 2007). Overall, our rainfall exclusion and warming treatments promoted soil drying, as shown in Escolar and others (2012) and Maestre and others (2013). A detailed analysis of soil moisture changes after rainfall events in our experiment showed how biocrusts increased water gains after rainfall events but enhanced soil desiccation after rainfall pulses (Lafuente and others 2018). Thus, the reductions in water availability due to our climate change treatments, particularly in areas with high biocrust cover, might explain the decreases in N_2O emissions observed in these plots (Fig. S2). Alternatively, nutrient availability is also often highlighted as a key driver of dryland N_2O fluxes (Dalal and Allen 2008; Dijkstra and others 2013). Under aerobic conditions and high availability of N substrate (i.e. NH_4^+), nitrification is expected to dominate over denitrification (Weier and others 1993; Dalal and others 2003), which

have been reported to lead to an accumulation of inorganic N forms in global drylands (Delgado-Baquerizo and others 2016). More importantly, we have found in our experiment that warming + rainfall exclusion treatments often lead to an accumulation of inorganic N in low (not in high) biocrust cover plots (Delgado-Baquerizo and others 2014). Similarly, previous studies at our experimental site have found a higher potential nitrification rate and available NO_3^- in bare soil areas compared to areas with well-developed biocrusts (Castillo-Monroy and others 2010), where DON is the dominant N form (Delgado-Baquerizo and others 2010). Thus, in drylands, having a well-developed biocrust community could be linked to a lower accumulation of inorganic N (Delgado-Baquerizo and others 2014), therefore limiting the availability of substrate for the denitrification process and ultimately reducing N_2O fluxes to the atmosphere from incomplete denitrification leaks (Dalal and Allen 2008).

It is important to highlight the importance of the selected denitrification gene studied. Under aerobic conditions, nitrification produces N_2O as a by-product (Bremner 1997; Canfield and others 2010), a process that is expected to be important in drylands given their reported relatively high mineralization rates. However, denitrification is an anaerobic multistep process that also produces N_2O (Firestone and Davidson 1989). Anaerobic soils are not dominant in drylands, but favourable conditions for denitrification can be created in soil aggregates or after precipitation pulses (Austin and others 2004; Ley and others 2018). The last step of the denitrification pathway consists on the conversion of N_2O into N_2, a step catalysed by the nitrous oxide reductase codified by the functional gene *nosZ* (Philippot and others 2007). Consequently, the *nosZ* gene has been used to estimate N_2O fluxes (Powell and others 2015). Our climate change treatments had no detectable effects on this gene regardless the initial biocrust cover considered. However, the abundance of the *nosZ* gene tended to increase in the warming and rainfall exclusion treatments only in high biocrust

248 cover plots (Fig. 2a, b). This may also help explain, at least partially, the average lower rates of N_2O observed in these plots
249 throughout the whole study. However, we cannot obviate that (i) we have evaluated functional genes at DNA level, and consequently
250 we cannot know whether this gene is being expressed or not; and (ii) the primers used, which fail to amplify *nosZ* clade II gene (Jones
251 and others 2013), have recently been described to be abundant in soils and thus an important contributor to N_2O fluxes (Domeignoz-
252 Horta and others 2016; Stein 2017; Hallin and others 2018).

253 Our climate change treatments consistently and relatively reduced CH_4 uptake, as found in another study carried out in a
254 semiarid grassland (Dijkstra and others 2013). Methane oxidation requires gas diffusivity to provide atmospheric CH_4 to soil
255 methanotrophs, a step catalysed by the CH_4 monooxygenase codified by the *pmoA* gene (Dalal and Allen 2008). In more mesic
256 ecosystems, decreased soil moisture would improve gas diffusivity, increase soil aeration and CH_4 oxidation. However, drylands are
257 water limited ecosystems, so further reductions in soil moisture by our climate change treatments might have limited the activity of
258 CH_4 oxidizing bacteria (Schnell and King 1996; Galbally and others 2008; Sullivan and others 2013) (Figs. S2 and S3). Similarly,
259 increased temperatures have been described previously to drive changes in the community composition of CH_4 oxidizing bacteria
260 (Mohanty and others 2007), which can also help to explain the differences in CH_4 uptake observed among treatments (Nazaries and
261 others 2013). Interestingly, we observed a positive correlation between changes in biocrust cover during the lifetime of our experiment
262 and CH_4 uptake. Put simply, the loss of cover through time observed in high biocrust cover plots (Ladrón de Guevara and others 2018)
263 was linked to decreases in CH_4 uptake (Fig. S7). Methane oxidation is very sensitive to changes in temperature and more importantly
264 in moisture, changes related to water stress and gas diffusivity (Smith and others 2000). Thus, it is likely that the known changes

exerted by biocrusts in soil properties and processes (Barger and others 2016; Chamizo and others 2016; Weber and others 2016), might have improved the environmental conditions (e.g. mitigating water and heat stress) for methanotrophs or changed the CH_4 oxidising bacterial community, affecting CH_4 uptake, even in deeper layers where most CH_4 uptake occurs (Butterbach-Bahl and Papen 2002).

The abundance of the *pmoA* gene was higher in high than in low biocrust cover plots. The well-known positive impacts of biocrusts on soil fertility (Weber and others 2016) could underlie this increase in microbial abundance (Maestre and others 2011; Barger and others 2016), which in turn might have contributed, at least partially, to increase the overall CH_4 uptake observed during the entire duration of this study (Le Mer and Roger 2001). However, and in contrast with a previous study carried out in an Australian forest that found correlated gene abundances and GHG emissions (Martins and others 2016), we could not find a relationship between the overall abundance of the *pmoA* gene and overall CH_4 uptake. Methane uptake depends on the balance between gas diffusivity and metabolic stress (Luo and others 2013). Thus, our results can be the consequence of microbial activity limitation due to water stress. Indeed, in low biocrust cover plots, we detected an increase in *pmoA* abundance in the warming + rainfall exclusion treatment (Fig. 2c). Despite such increase, this treatment did not show enhanced CH_4 uptake, which supports that reductions in soil moisture could have limited microbial metabolism. Alternatively, we cannot discard that the interference of soil NH_4^+, which competes with CH_4 for the methane monooxygenase (King and Schnell 1994), could be behind the observed lack of correlation between *pmoA* abundance and CH_4 uptake.

Together, our findings highlight how biocrusts are essential regulators of soil-atmosphere N_2O and CH_4 fluxes and their responses to simulated climate change, directly and indirectly by improving soil environmental conditions (i.e. reducing water and heat stress) for N_2O reducers and methanotrophs. They also show that functional microbial abundance (i.e. *nosZ* and *pmoA* carrying bacteria) can also be highly variable in time, providing evidence for seasonal patterns in these functionally important bacterial communities. Our results also illustrate how biocrusts affect temporal patterns in the fluxes of N_2O and CH_4 and associated functional genes. On average, the "biocrust legacy" reduced the rate of N_2O emissions, increased the rate of CH_4 uptake and increased the abundance of both *nosZ* and *pmoA* genes. More importantly, biocrusts mitigated the reductions in CH_4 uptakes observed under the combination of warming and rainfall exclusion treatments. Our findings emphasize the importance of well-developed biocrust communities to mitigate the impacts of warming and altered rainfall patterns on the emission of GHG fluxes from dryland soils, and thus the need to preserve them to minimize the negative consequences of ongoing climate change and to maintain ecosystem functioning in a warmer and drier world.

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Table 1. Linear mix model of the effect of climate change treatments on N_2O and CH_4 fluxes (n = 7) and functional gene abundances (n=5). The rate of change in the biocrust cover over time (ΔBSC, in %) has been included in the models as a covariate to control for the observed changes in biocrust cover since the setup of the plots in 2008. WA = warming, RE = rainfall exclusion, BSC = biocrust cover, Num = Numerator degrees of freedom and Den = denominator degrees of freedom.

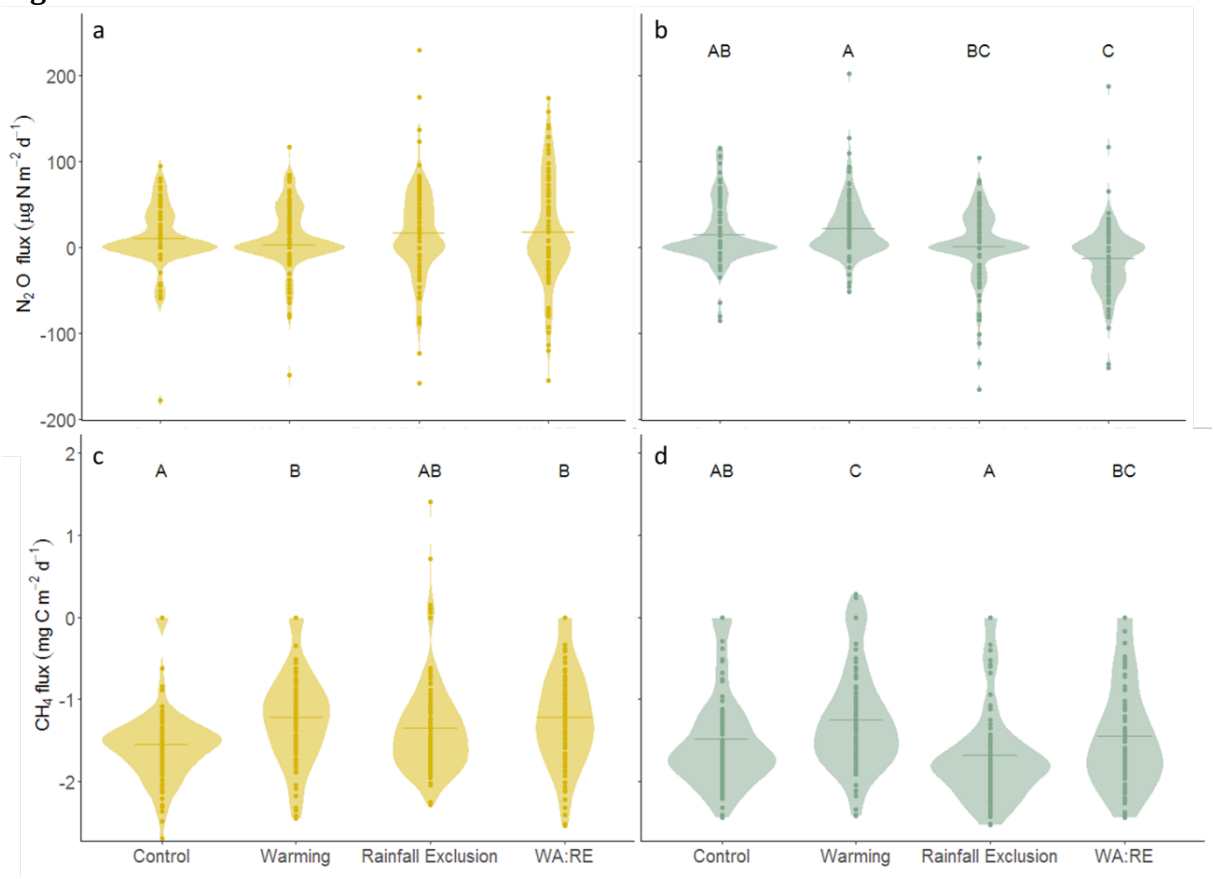
	N_2O				CH_4				*nosZ*				*pmoA*			
	Num	Den	F	*P*	Num	Den	F	*P*	Num	Den	F	*P*	Num	Den	F	*P*
Warming	1	51.19	0.55	0.461	1	52.96	6.88	**0.011**	1	31.60	0.61	0.440	1	32.13	3.30	0.079
Rainfall Exclusion	1	47.60	1.56	0.218	1	48.45	0.42	0.522	1	30.42	0.10	0.755	1	30.66	0.00	0.986
BSC	1	51.29	1.37	0.248	1	53.08	9.22	**0.004**	1	31.10	9.56	**0.004**	1	31.68	12.87	**0.001**
WA:RE	1	47.60	0.53	0.471	1	48.45	1.28	0.263	1	30.37	1.81	0.188	1	30.67	0.03	0.854
WA:BSC	1	50.13	0.02	0.896	1	51.61	0.66	0.419	1	30.66	0.10	0.753	1	31.08	0.94	0.339
RE:BSC	1	47.67	12.02	**0.001**	1	48.55	7.78	**0.007**	1	30.10	0.00	0.952	1	30.39	0.07	0.793
WA:RE:BSC	1	47.64	1.89	0.176	1	48.51	1.48	0.230	1	30.08	6.20	**0.018**	1	30.40	1.23	0.276
ΔBSC	1	72.47	0.16	0.686	1	84.66	2.66	0.106	1	40.22	0.23	0.637	1	41.33	0.62	0.436

Figure legends

Figure 1. N_2O (a, b) and CH_4 (c,d) fluxes estimated in areas with low (left) and high (right) initial biocrust cover across the climate change treatments evaluated, the horizontal line shows the mean (*n*=98). The width of shaded area in the violin plot represents the kernel probability density (proportion of the data located there). Different letters indicate differences in pairwise comparisons among treatments by differences of least square means ($P<0.05$). WA:RE = warming and rainfall exclusion combined.

Figure 2. Log-transformed abundances of *nosZ* (a,b) and *pmoA* genes (c,d) in areas with low (left) and high (right) initial biocrust cover across the climate change treatments evaluated, the horizontal line shows the mean (*n*=25). The width of shaded area in the violin plot represents the kernel probability density (proportion of the data located there). Different letters indicate differences in pairwise comparisons among treatments by differences of least square means ($P<0.05$). WA:RE = warming and rainfall exclusion combined.

Figure 1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N_2O flux ($\mu g\ N\ m^{-2}\ d^{-1}$)
CH_4 flux ($mg\ C\ m^{-2}\ d^{-1}$)
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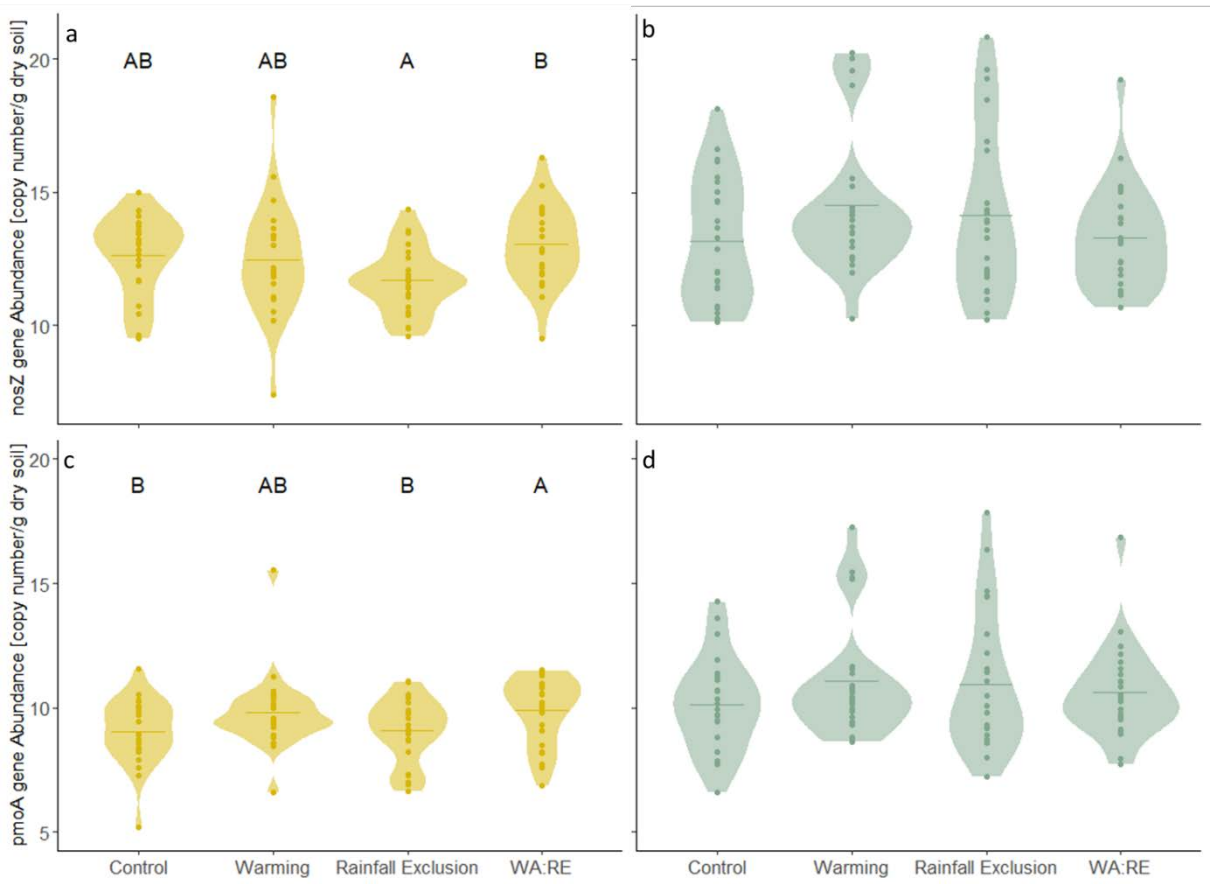
Rainfall Exclusion
WA:RE
Control
Warming
Rainfall Exclusion
WA:RE

493 **Figure 2**



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