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Climate change effects on soil N and P cycling and greenhouse gas fluxes in cork oak forests of southern Spain

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Abstract

English

The Mediterranean basin is a culturally and biodiversity rich region that is expected to suffer some of the worst effects of predicted climate change with higher temperatures, significant decreases in precipitation, and more extreme climatic events. Los Alcornocales natural park in southern Spain is one of the largest protected cork oak forests in the region, with great ecological, cultural, and economic value. Its longstanding forests provide many ecosystem services but are threatened by several interacting stressors such as human disturbances, climatic changes, pathogens, and pests. Thus, we aimed to study how key ecosystem processes, soil nitrogen and phosphorus cycling and greenhouse gas fluxes, would be impacted by these predicted climatic changes. Treatments were established that increased temperature by 2-3°C, reduced rainfall by ~30%, and a combination treatment of both. We found that all soil nutrients were significantly impacted by reductions in rainfall, with altered seasonal patterns of production. Greenhouse gases fluxes were also impacted on a seasonal basis, with nitrous oxide emissions significantly increased overall by temperature treatments, carbon dioxide exhibiting variable responses, and methane fixation rates reduced under climate change conditions. We conclude that soil nutrient cycles and greenhouse gas fluxes were significantly altered by our treatments, and that climate change will have serious impacts on ecosystem functionality.

Portuguese

A bacia do Mediterrâneo é uma região rica em cultura e biodiversidade que é provável que sofra alguns dos piores efeitos das mudanças climáticas, incluindo temperaturas mais altas, diminuições significativas na precipitação e eventos climáticos mais extremos. O parque natural “Los Alcornocales”, no sul da Espanha, é uma das maiores florestas de sobreiro protegidas do Mediterrâneo, com um grande valor ecológico, cultural e econômico. As suas florestas fornecem muitos serviços ecossistêmicos, mas estão ameaçadas por vários fatores de estresse interativos, como perturbações antropogénicas, mudanças climáticas, patógenos e pragas. O objetivo de esta tese é estudar como processos ecossistêmicos chave (especificamente a produção de nitrogênio e fósforo no solo e os fluxos de gases de efeito estufa) vão ser impactados por essas mudanças na temperatura e na precipitação previstas pela maioria dos modelos climáticos. Para alcançar os nossos objetivos, foram estabelecidos em parcelas naturais sistemas de manipulação climática que aumentaram a temperatura do solo em 2-3°C, reduziram a precipitação em ~ 30% (alem de um tratamento combinado dos dois anteriores). Os nossos resultados mostram que a produção de todos os nutrientes do solo considerados foi significativamente influenciada pelas reduções na precipitação, com padrões sazonais de produção também alterados. Os fluxos de gases de efeito estufa mostraram uma grande variabilidade sazonal. Apesar disso, as emissões de óxido nitroso aumentaram significativamente com o aumento datemperatura[41] , e as taxas de fixação de metano foram reduzidas sob condições de mudanças climáticas. Estes resultados permitem-nos concluir que as mudanças previstas na temperatura e na precipitação provavelmente resultaram em alterações significativas na capacidade destas florestas para disponibilizar nutrientes e trocar gases de efeito estufa com a atmosfera.

Keywords: N cycling, P cycling, greenhouse gases, climate change, Mediterranean

List of Abbreviations

CO_2	Carbon dioxide
CH_4	Methane
GHG	Greenhouse gas
N	Nitrogen
N_2O	Nitrous oxide
NH_4	Ammonium
NH_4NO_3	Total inorganic nitrogen
NO_3	Nitrate
OTC	Open top chamber
P	Phosphorus
RE	Rain exclusion
Temp	Temperature

1 Introduction

The current climatic changes that we are experiencing are unprecedented and are expected to continue to intensify across the world in the coming years. These changes are likely to have important implications for ecosystem functioning, and consequently, human livelihoods (IPCC, 2018). This is particularly true in the case of the Mediterranean region in Europe, where 472 million called the region home in 2010, and is predicted to reach 572 million by 2030 (UN Environment Program, 2017). The Mediterranean basin may only cover 2 % of the Earth's surface, but it is one of 34 biodiversity hotspots based on the multitude of plant and animal species that inhabit it, including a high number of endemic species (Gauquelin et al., 2018; European Commission, 2009).

This culturally and biodiversity rich region is expected to suffer some of the worst effects of forecasted climate change. Average temperatures in the region have already risen by 1.4°C since the pre-industrial era, and drought severity has intensified with a loss of 15.6% precipitation with an additional 7.4% evaporative demand in the last five decades (Avila et al. 2019; Vicente-Serrano et al. 2014). The IPCC forecasts that southern Europe will continue to experience higher temperatures than any other region in Europe, with significant decreases in precipitation and more extreme climatic events. Droughts are expected to lengthen, and soil moisture is predicted to significantly decrease in response to the higher temperatures. (Grillakis, 2019; Cramer et al., 2018; Tueling, 2018; Ozturk et al., 2015; Valentini et al., 2014).

Increasing temperatures and loss of soil moisture by reductions in rainfall both have known impacts on soil functioning and plant growth. Thus, reductions in soil moisture limits plant growth and nutrient uptake, and many soil processes are dictated by complex microbial communities which are very responsive to changes in temperature and soil moisture, with studies showing reductions in soil enzymatic activity or community composition and abundance under drought conditions (Preece et al., 2019; Bastida et al., 2019; Ren et al., 2018; Naylor et al., 2018; Xu & Yuan, 2017; Meisner et al., 2013; Auyeung et al., 2013 Sardans & Peñuelas, 2008). This can lead to changes in the rate and timing at which bioavailable soil nutrients are transformed (Homyak et al., 2017; Matías et al., 2010; Sardans & Peñuelas, 2005), and in the rate of emission or fixation of greenhouse gases (Smith et al., 2018; Oertel et al., 2016; Martins et al., 2015). However, as these reactions have large spatial variability due to differences in soil characteristics and local climatic dynamic, the effects of climate change are likely to differ across localities (Reys-Garcia et al., 2016), and we are still far to fully understand how climate will impact ecosystem functioning in many areas of the planet, including Mediterranean forests.

Therefore, this study was conducted in Los Alcornocales natural park, a protected cork-oak forest in southern Spain with great ecological, cultural, and economic value. Its long-standing forests provide many ecosystem services but are threatened by several interacting stressors such as human disturbances, climatic changes, pathogens, and pests. (Costa, 2016; Kim et al., 2017; Moricca et al., 2016). These oak forests have provided economic stability for local communities for at least the past two centuries through cork-oak production, with 16,000 metric tons of cork being harvested from this area annually (Aronson et al., 2009). Other resources include are lowland pasture

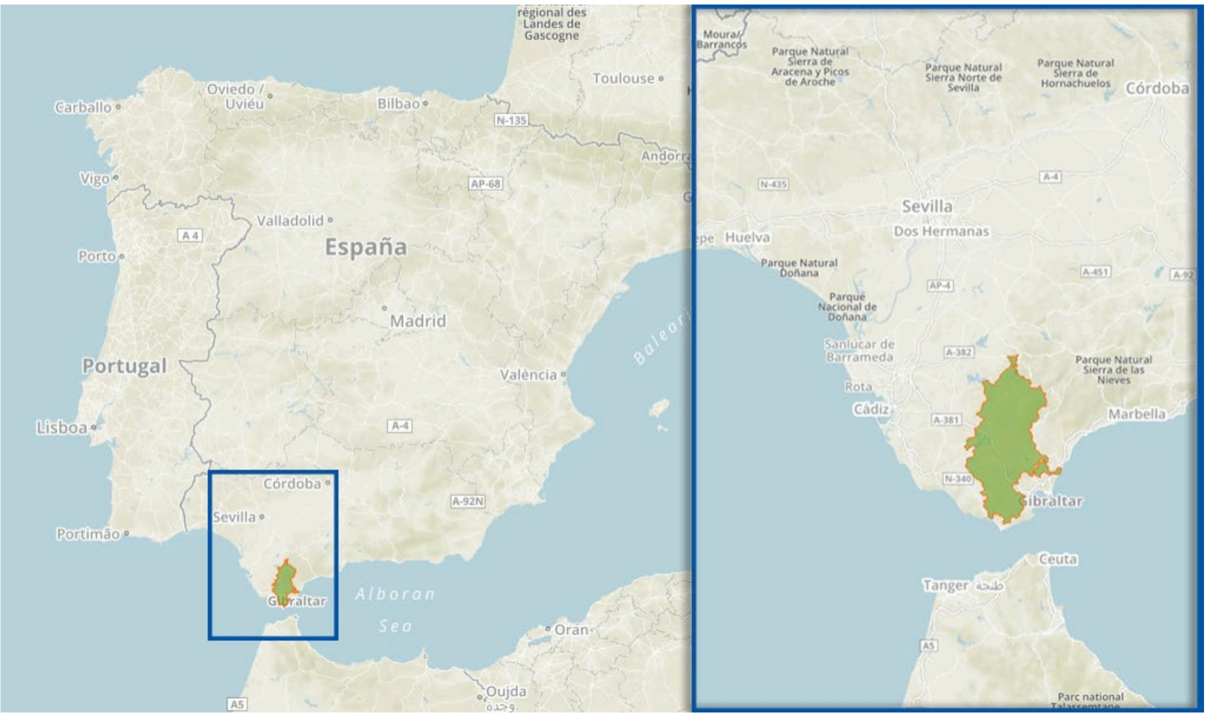

Figure 1: Location of Los Alcornocales park. Map imagery taken from Protected Planet (2014).

for free-range cattle, wild game for hunting such as red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), and many non-timber forest products (Costa, 2016; Kim et al., 2017, Aronson et al., 2009). Besides provisioning ecosystem services, the forest also provides regulating services such as soil conservation, water retention, watershed protection, erosion control, fire risk prevention, carbon sequestration (WWF, 2004; Aronson et al., 2009). Climate change is already directly impacting these forests with decreased precipitation and increased temperature causing physiological stress to the trees, causing limited nutrient uptake, stem mortality and seedling death (Liu et al., 2018; Costa, 2016; Pressarakli, 1999). Invasive pathogens such as *Phytophthora cinnamomi,* are spread much more quickly under increasing temperatures and longer warm seasons, and limit nutrient uptake in cork oaks causing further stress and extensive die-off (Homet et al., 2019; Gómez-Aparicio et al., 2017; Kim et al., 2017; Avila et al., 2016). Thus, the interacting challenges that these valuable forests are facing make understanding the effects of predicted climate change on soil nutrient cycling and greenhouse gases emissions even more imperative.

1.1 Background

While these forests are already adapted to nutrient poor soils, further reductions or changes in soil nutrients could be detrimental (Liu, 2010). Soil nitrogen (N) and phosphorus (P) are two of the most important soil nutrients for microorganisms and vegetation, and the available forms of these nutrients are largely produced in the soil by complex communities of soil microbes (Bardgett & van der Putten, 2014). Many of the processes involving soil nutrients are also interconnected with the exchange of greenhouse gases (GHG) between the soil and the atmosphere, so the determination of climate change effects on these soil processes are integral (Walkiewicz & Brzezińska, 2019; Oretel et al., 2016; Veraart et al., 2015; Singh, 2011; Leifeld, 2007). Decreases in soil moisture, due to decreases in precipitation, have been shown to suppress microbial activity and to change or decrease soil microbial communities that facilitate the soil nutrient cycling processes (Ren et al., 2018; Homyak et al., 2017; Bérard et al., 2011). Meanwhile, increases in temperatures are shown to stimulate microbial communities, which could at least partially compensate the effect of decreases in precipitation. Similarly, increases in temperature have also been shown to increase the soil to atmosphere greenhouse gases fluxes (Duan et al., 2018; Schaufler et al., 2010). Thus, forecasted increases in temperature could at least partially compensate the negative effects of decreasing soil moisture on soil functioning, at least in the short term (Pold & DeAngelis, 2013). However, increasing soil temperature also increases soil evapotranspiration, which could exacerbate the negative impacts of decreasing precipitation in water-limited ecosystems (Samaniego et al., 2018; Vicente-Serrano et al., 2014)

1.1.1 Soil nutrient cycling

The soil nitrogen cycle is composed of four main steps: fixation, mineralization, nitrification, and denitrification (Figure 2). Fixation is the process by which atmospheric gaseous dinitrogen (N2) is fixed in the soil by diazotrophic bacteria, either free-living in the soil substrate or those that have a symbiotic relationship with plant roots. These bacteria use nitrogenase enzymes to transform N_2, which is metabolically useless to all but a few microorganisms, to ammonia, which can metabolized by most organisms and incorporated into biological structures (also known as immobilization). Mineralization occurs thereafter, where the previously fixed and immobile ammonia is

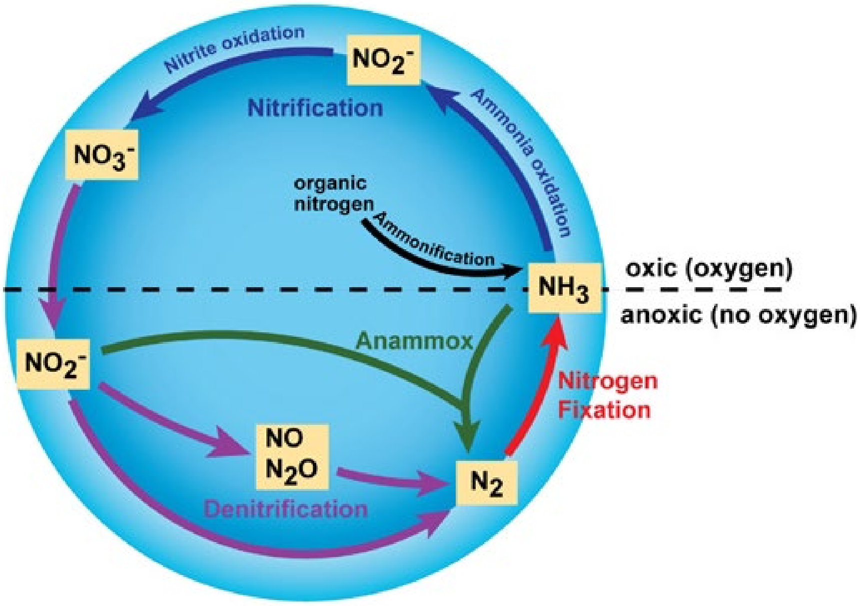

Figure 2: Simplified diagram of nitrogen cycle, from Berhanrd, 2010.

then oxidized to ammonium (NH_4^+), becoming again bioavailable to plants and soil microorganisms. The rates of mineralization vary with soil temperature, moisture and the amount of oxygen in the soil (Fowler et al., 2013; Singh, 2011; Bernhard, 2010; Johnson et al., 2005). After mineralization, ammonium undergoes a two-step conversion by soil-living bacteria which obtain energy by converting ammonium to nitrite (NO_2^-), and then to nitrate (NO_3^-) under a process known as nitrification. Nitrification is most rapid when soil is warm (between 28°C and 36°C), moist, and well-aerated (Bernhard, 2010; Johnson et al., 2005; IPNI, 2004a). Nitrifying bacteria are especially sensitive to changes in soil water content as it affects the equilibrium between soluble and exchangeable ammonium, the concentration of salts, and oxygen availability (IPNI, 2004a). The final step of the nitrogen cycle is denitrification, where nitrate is reduced progressively into gaseous forms of nitrogen gas (N_2), nitric oxide (NO), or nitrous oxide (N_2O) under primarily anaerobic conditions. The determination of the ratios of these outputs depends a lot on the soil conditions, particularly soil water content. Denitrification can occur at low rates under aerobic conditions; however, it is the dominating process under anaerobic conditions. At 60% waterfilled pore space nitrous oxide is the primary byproduct, while dinitrogen gas becomes the primary product after about 75% water-filled pore space (van Groenigen et al., 2015; GRDC, 2014; Singh, 2011; Bernhard, 2010; IPNI, 2004b). Temperatures have less impact on denitrification rate than soil moisture, but ideal temperatures are between 27-38°C (GRDC, 2014)

While phosphorous usually only makes up about 0.2% of plant weight, it is also a necessary nutrient for plant growth, maturity, and reproduction. This is because phosphate is an important part of adenosine triphosphate (ATP), which is used in many biochemical processes in plants and allows them to extract nutrients from soils. It is necessary for energy transport, cellular structures, and nucleic acids (Margalef et al., 2017; Hyland et al., 2005). Bioavailable, or inorganic, phosphorous is transformed through the mineralization of organic P to $H_2PO_4^-$ or HPO_4^{2-}(Figure 3) (Hyland et al., 2005). Mineralization is made possible by phosphatase enzymes that are produced by bacteria, fungi, and plant roots (Maragalef et al, 2017). It has been shown that microbial communities responsible for phosphorus mineralization have sensitivity to temperature and soil moisture changes, with decreases in inorganic production rates under drought conditions and increases of temperature above 40°C (Brödlin et al.,2019; Maragalef et al, 2017; Hinojosa et al., 2012; Butterly et al., 2011; Sardans & Peñuelas, 2007, 2004).

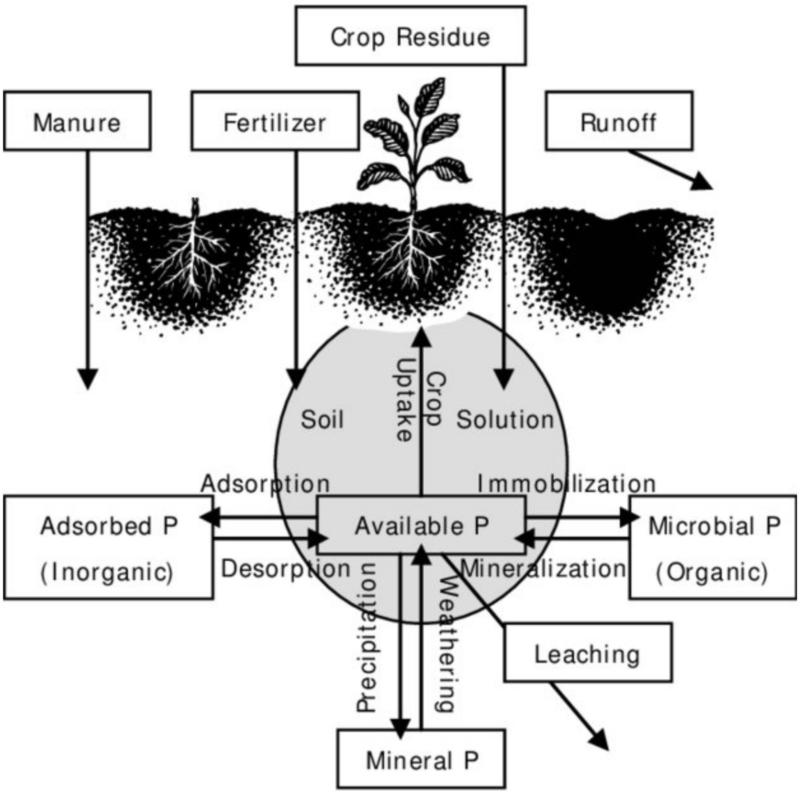

Figure 3: Simplified phosphorus cycle, from Hyland et al., 2005.

1.1.2 Greenhouse gases

Greenhouse gases are major contributors to global warming, and investigating their sources is important to properly inform global climate models for accuracy, and for management strategies to minimize their output (Oretal et al., 2016). Soils have the potential to emit GHG through transformational processes by soil microbial communities, but also to act as 'sinks' by capturing organic forms of these elements. These dynamics depend on the biotic and abiotic soil characteristics of each ecosystem. The biotic sources of greenhouse gases are driven by dynamic and complex processes and vary with environmental conditions such as plant and microbial community structure, soil organic matter, ammonium and nitrate concentrations, soil water content, and temperature (Shvaleva et al., 2011; Leifeld et al., 2007). Therefore, the fluxes of greenhouse gases can be significantly impacted by changes in climate conditions. In this study we consider the flux dynamics between soil and atmosphere for the three most detrimental greenhouse gases: carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4).

Carbon dioxide is the most notorious greenhouse gas, and much of the anthropogenically caused global warming is attributed to the increases in CO_2 emissions from human activities over the past century. Carbon dioxide is emitted from soil through microbial respiration (anaerobic and aerobic) and root respiration (Rastogi et al., 2002). Emission rates of CO_2 are dependent on a number of factors such as soil texture, pH, available organic carbon, and nitrogen content of the soil, but temperature and soil moisture hold the most sway over the decomposition rates that result in CO_2 emissions (Smith et al., 2018; Rastogi et al., 2002).

Nitrous oxide gas is a long-lived relatively active greenhouse gas with an atmospheric lifetime of about 120 years. Its global warming potential is 265 times more powerful than CO_2 and is responsible for about 6% of global warming (Myhre et al., 2013; Ussiri & Lal, 2013). It also plays a significant role in the depletion of stratospheric ozone (Ussiri & Lal, 2013). Nitrous oxide is emitted by soils during nitrification (oxidation of NH_4^+ to NO_2^- and NO_3^-) and denitrification (reduction of NO_3 to N_2O and N_2). However, its mainly produced by denitrification under anaerobic conditions, when soil water content is above 50% (Oertel et al., 2016; Groenigen et al.,2015). The availability of inorganic N together with organic matter (heterotrophic transformation) or carbon dioxide and oxygen (autotrophic transformation) also determines N_2O production rates (Leifeld, 2007). Nitrifier denitrification is a relatively newly discovered source of N_2O, and there is a lot of discussion on how much is actually emitted under this process, so it's significance as a source of N_2O is still under discussion, especially in dryland ecosystems (Smith et al., 2018; Wrage-Mönnig et al., 2018; Oretel et al., 2016; Kool et al., 2011.)

The concentration of CH_4 in the atmosphere has more than doubled since the preindustrial era, with soils contributing about 40% of total emissions (Smith et al., 2018). Methane has a global warming potential of 28 times that of carbon dioxide, with an atmospheric life time of 12.4 years (Myhre et al., 2013). In anerobic soils, methane is produced through methanogenesis, and is consumed by methanotrophic microorganisms that use O_2 and CH_4 for their metabolism under aerobic conditions (Oretel et al., 2016; Smith et al., 2018; Leifeld, 2007). While most atmospheric methane is destroyed by oxidation in the atmosphere, 5–10% is diffused into aerated topsoils, where it is oxidized by soil microorganisms, this diffusion is primarily influenced by soil moisture, texture and bulk density (Shvaleva et al., 2014). Soils are CH_4 sinks under aerobic conditions, and sources in anerobic conditions. In the Mediterranean, forest soils are typically sinks of methane, not sources (Oertel et al., 2016; Shvaleva et al., 2011). However, changes in soil moisture and precipitation, as well as the forecasted more sporadic but intense precipitation events, are likely affect the net flux of CH_4 between the soil and the atmosphere in these areas.

1.2 Objective

In this study, we aimed use a manipulative field experiment to investigate the changes induced by increases in temperature and decreases in precipitation (and the combination of both) on the cycling of soil nitrogen and phosphorus, and on the fluxes of carbon dioxide, nitrous oxide, and methane in the soils of Los Alcornocales natural park. Many of these processes have been shown to be sensitive to forecasted climate changes. However, changes in ecosystem functioning are the result of complex interactions between these key processes and site-specific environmental conditions, making it difficult to forecast future scenarios based on single-factor studies or on studies carried out in other systems. Most of these studies are conducted in more temperate or tropical ecosystems, with either observational or laboratory-based manipulations that typically only include one factor. This manipulative study is novel in its field-based, full-factorial design that is inclusive of seasonal variation over one full year of sampling. Due to the variation between seasons, which is characteristic of

Mediterranean ecosystems, climate change may have different impacts on soil functioning at different times of year, and therefore this study offers insight to these interacting dynamics.

2 Material and Methods

2.1 Study site

This study was carried out at Los Alcornocales Natural Park in Southern Spain, known to be an ecological hotspot and one of the largest cork-oak forests in Western Europe, covering 170,025 hectares (Aila et al., 2017; Jiminez-Chacon et al., 2018). The climate of the region is characterized as sub-humid Mediterranean with hot, dry summers and mild, wet winters. Temperatures range between 7-10°C in winter, and 22-26°C in summer, with an annual average temperature of 15.7°C (Avila, 2017, 2019). Due to its proximity to the Mediterranean Sea, and the predominant western winds, there is a relatively high degree of humidity, with an average annual rainfall of 748-1100mm, 95% of it occurring between October and May (Jiminez, 2018; Avila, 2018; Aponte Matais et al., 2014). The study site was established in the drier lowlands of the park (36◦34′07.5″, N 5◦32′08.3″ W), where forest grows in acidic, nutrient-poor, sandy soils and is composed of two dominant tree types: *Quercus suber L.* and *Olea europaea var. sylvestris Brot* (Table 1) (Avila, 2017).

Table 1:: Soil physical properties of study area, taken from Gómez-Aparicio et al (2017), where values represent median [P10–P90, 10th and 90th percentiles] for 49 sampled points regularly distributed in a 70 m × 70 m square plot at each site.

Soil chemical and physical properties	
Total C (mm/g)	45.15 [35.17,55.70]
Total N (mm/g)	2.30 [1.92,2.90]
Available P (mm/Kg)	7.50 [6.40-9.81]
Ca (mm/Kg)	684.32 [467.4-865.4]
Mg (mm/Kg)	142.23 [91.47-201.81]
K (mm/Kg)	117.63 [81.77-146.18]
Clay content (%)	12.84 [10.03-16.71]
Sand content (%)	73.59 [69.84-78.36]

2.2 Experimental design

2.2.1 Establishment of Treatments

In 2015, six experimental plots (20 x 15 m) were established within the study site: three that included rain exclusion (RE) structures and three control. Each RE plot was located adjacently to a control plot to minimize edaphic variations between the two treatments. The three paired blocks were separated by a minimum of 200m. In each plot, 3 adult individuals per tree species were selected and characterized (36 individuals in total). Rainfall exclusions were created by suspending plastic white PVC channels without ultraviolet filter of 2.5 cm in diameter over the surface of the forest floor at a minimum height of one meter to minimize the microclimatic effect on the soil (Figure 4B). The structure was supported by a network of iron supports to ensure the stability of the structure in the long term and allow its slope to be adjusted to a minimum of 5% for the correct evacuation of intercepted water. Each RE plot had 24 rows of channels separated from each other by approximately 70 cm. In the control plots, a similar structure was built, but with the channels upside down so that possible microclimatic effects of the structure were reproduced but without intercepting rainwater. To avoid edge effects no measurements were made in the outer meter of the perimeter of each plot. All plots were fenced to avoid the passage of large herbivores that could damage the infrastructure. In addition to the rainfall exclusion treatment, a temperature increase treatment with two levels (ambient and + 2-3 °C) was set up next to each selected tree since 2016. A 2-3 °C increase was

Figure 4: A: Example of open top chamber (OTC) (Wahren, 2019) B:Rain exclusion setup in Los Alcornacales (Jiméinez et al 2018)

achieved by using 40 × 50 × 32 cm hexagonal open top chambers (OTCs, Figure 4A). Open top chambers are a well-established method to simulate climate warming and have been successfully used to study the effect of warming from arctic and tundra plants (Yang et al, 2018; Bokhorst et al 2013) to tropical grasslands (Buhrmann et al, 2016). The OTCs were made of methacrylate and elevated 5 cm from the surface to avoid overheating. Thus, our full-factorial experimental design comprised a total of four treatments: control, rain exclusion, temperature, and a combined treatment of temperature and rain exclusion (T+D); as well as 18 sampling points per treatment (72 sampling points in total). All sampling points were sampled in summer, fall, and winter of 2017, and in spring of 2018 to account for interseasonal variability. Winter 2017 samples were omitted for soil nutrient measurements, due to sampling error.

2.3 Field procedures

2.3.1 Soil Nutrient Sampling

To assess soil total inorganic nitrogen (N) and phosphorus (P) availability, we used ion exchange membranes (IEMs). The resins used on IEMs exhibit emulate plant root surfaces, taking into account the nutrient diffusion capacity in soil. Studies have shown that the ion concentrations in IEM extracts correlate to plant uptake of nutrients, thus, these membranes have been established as an effective method for the analysis of soil nutrients availability and production (Duran et al, 2013; Qian, Schoenau, & Ziadi, 2008, Rieman & Walton, 1970). For each sampling point, two sets of cationic and anionic exchange membranes (3cm x 3cm) were attached to 30 cm rulers at 15 cm and 30 cm depth using acrylic adhesive, vertically placed in the soil leaving the first 5 cm out for removal (therefore the actual depths were 10 and 25 cm), and incubated for one month. After removal, they were kept in plastic bags in a cool dry place until lab processing.

2.3.2 Greenhouse Gases Sampling

We used the static chamber technique to measure the in-situ greenhouse gases (GHG) exchange between the soil and the atmosphere. A PVC ring (diameter = 20 cm, height = 10 cm) was inserted approximately 5 cm into the ground at the start of the experiment at each sampling point. Immediately before each measurement, a 20 cm diameter, 9 cm high PVC chamber was placed on top of each ring and sealed with a rubber band. Each chamber had a sampling port in the top center of the chamber 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 hypodermic needle attached to a polypropylene syringe, transferred to 22 ml pre-vacuumed vials and kept at room temperature until analysis. Measurements were taken in a randomized design between 10 a.m. and 12 p.m. to minimize sharp temperature variation effects on GHG sampling.

2.4 Lab Procedures

2.4.1 Soil Nutrient Analysis

The IEMs were separated from the rulers, washed with distilled water, and air dried. Then, NH_4^+-N, NO_3^--N and PO_4^{-3}-P were extracted from the membranes following Rodríguez et al. (2009). In summary, IEMs were cut in half, placed in 50 ml falcon tubes, and shaken for 60 minutes at 200 rpms in 30 ml of 1M KCl. These extracts were immediately filtered through standard lab filters (125mm diameter ashless filter papers, GE Healthcare & Filter Lab) and kept in the freezer (-20°C) until analysis. The amount of inorganic N and P in the extracts was analyzed by colorimetry using a microplate reader (Sims et al., 1995). In the case of N, we used the indophenol blue method, while P was measured using the molybdenum blue method (Allen et al., 1986). Concentrations (ppm) of NH_4^+-N, NO_3^--N and PO_4^{-3}-P in the extracts were estimated with the help of standard curves. The ppm values were then converted to a rate of µg of nutrient per square centimeter per day by using the following formula:

$$r = \frac{\frac{PV}{S}}{t}$$

Where the rate (*r)* equals the concentration of the sample in ppm (P) per the volume of KCl used to extract the sample in ml (V), divided by the surface area of the IEM in cm^2 (S), divided again by the incubation time in days (t). We then calculated soil total inorganic N (NH_4NO_3-N) as the sum of NH_4^+-N and NO_3^--N.

2.4.2 Greenhouse gases analysis

Gas samples were analyzed at the Instituto Superior de Agronomia (Lisbon), using gas chromatography. Specifically, by electron capture, thermal conductivity and flame ionization detection in the cases of N2O, CO2 and CH4, respectively. The carrier gas used was helium in all cases. The returned chromatograph values were used to calculate the concentration of each gas in the sample using the ideal gas law equation, and to estimate net gas fluxes between the soil and the atmosphere from the linear rate of change in gas concentration, the chamber internal volume, and the soil surface area (Durán et al., 2013)

2.5 Statistical Analysis

2.5.1 Data Exploration

First, boxplots were conducted for each independent variable to identify potential outliers (determined as values three times the interquartile range) and heteroscedasticity problems. No outliers were removed from soil nutrient data, while 16 outliers were removed from greenhouse gases data (six outliers from N_2O, five each from CO_2 and CH_4). Then, we constructed histograms to check for normality. Finally, we conducted an analyses of summary statistics to get means and standard errors for each variable among other statistics.

2.5.2 Statistical Models

As most nutrient variables were not normal and showed heteroscedasticity, we used generalized least squares (GLS) models. These models allow for non-normality and they allow the use of weight and correlation arguments to account for the typical temporal dependencies derived from the use of repeated measures (Hansen, 2007). Thus, we used temperature and rain exclusion treatments, as well as season as fixed factors. *Tree* was used as a correlating factor and an interaction between treatment and a season as weighted argument to correct heteroscedasticity.

To model the impact of our treatments and account for seasonal differences in soil greenhouse gas emissions, linear mixed-effect models (LMMs) were used. These models have been often used in ecological data to account for field variations, nested, and hierarchical relationships between variables in datasets, such as repeated measurements of an individual over time (Harrison et al, 2018). Here, we used *Tree* as a random factor, to incorporate the repeated sampling dependencies. Temperature and rain exclusion treatments were included as fixed factors, with two levels each. Season was also included as a fixed factor with four levels, to evaluate inter-seasonal variation. Residuals from the models were visually tested for normality and homoscedasticity. Methane and carbon dioxide were deemed normal and homoscedastic, while nitrous oxide was able to meet these assumptions after applying a square root transformation.

Model selection was carried out through a step-wise selection procedure using the Aikake information criterion (AIC) to select the best-fitted models. No-significant factors (high p-values) remained in the models when their interaction with other factors was significant. After determining the best model, an ANOVA was conducted to evaluate the significance of treatments and interactions. A summary of all models and factors used is found in Table 2, and all model results are found in Appendix I-VIII. All statistical tests were conducted using R statistical software (R Core Team, 2019). Linear mixed models were performed using the *lmer* package (Bates et al, 2015), and GLS models performed using the *nlme* package (Pinheiro et al., 2019).

Table 2: Summary of models used for ANOVA analysis for each variable.

	Dependent Variable	*Transformation*	*Model Type*	*Fixed Factors*	*Random Factor*	*Weights (GLS only)*
Greenhouse Gases	CO_2	None	LMM	T, R, S	Tree	
	NO_2	Square root	LMM	T, R, S		
	CH_4	None	LMM	T, R, S		
Soil Nutrients	NH_4	None	GLS	T, R, S		Tt, S
	NH_4 NO_3	None	GLS	T, R, S		Tt, S
	NO_3	None	GLS	T, R, S		Tt, S
	PO_4	None	GLS	T, R, S		Tt, S

Factor abbreviations: T=Temperature, R=Rain exclusion, S=Season, Tt=Treatment

3 Results

3.1 Soil nutrients availability

All surrogates of soil nutrient production rates were significantly affected by the rain exclusion treatment (Table 3). Specifically, soil ammonium (NH_4), total inorganic nitrogen (NH_4 + NO_3), and inorganic phosphate (PO_4) production rates were 42%, 9%, and 79% higher in the rain exclusion plots than in the control plots. Conversely, soil nitrate (NO_3) production was significantly lower in the rain exclusion plots than in the control. Neither temperature nor the combination of temperature and rainfall exclusion significantly affected any of the overall soil nutrient production rates. Our results also revealed a significant effect of season on all the soil nutrient production rates, with values consistently lower in summer than in fall and spring (Table 4) (Figure 5). We also found significant interactions between season and the rain exclusion treatment for the production of available ammonium, nitrate, and phosphate (Figure 5A,C,D). A significant interaction between our temperature treatment and season was also found for soil ammonium production (Figure 5A).

Our rain exclusion treatment decreased ammonium production by 30% in summer but increased it by 47% in fall. Ammonium production was also was found to be significantly increased by our temperature treatment in fall by 33%. Total inorganic N production was most impacted by the rain exclusion and combination treatments in spring, with a 45% and 13% increase, respectively, as compared to the control plots. In summer the rate was reduced by 39% in the rain exclusion treatment as compared to the control plots. Nitrate production was significantly impacted by the rain exclusion in summer, where the treatment decreased the rate by 50%. When considering a 90% confidence interval, nitrate also significantly increased (p=0.06) in the spring season by 22%. It was also revealed that the rate of phosphate production increased in fall in response to the rain exclusion treatment by six times the control rate, while remaining stable in spring and summer under all treatments.

Table 3: Summary ANOVA results conducted on GLS of soil nutrient cycling rates. Means are reported with standard errors in brackets. All mean rates are in $\mu g\ cm^2$ per day.

	Mean	ANOVA		
Ammonium		***D.F.***	**F**	***p***
Control	0.076 (0.005)	-	-	-
Temperature	0.092 (0.007)	1	1.91	0.167
RE	0.108 (0.011)	1	24.39	<0.0001*
T+D	0.097 (0.010)	1	0.26	0.608
Season	-	2	86.81	<0.0001*
Temp:Season	-	2	6.02	0.0026*
RE:Season	-	2	8.53	0.0002*
T+D:Season	-	2	1.98	0.1399
Nitrate				
Control	0.150 (0.018)	-	-	-
Temperature	0.156 (0.021)	1	3.05	0.081
RE	0.134 (0.013)	1	23.72	<0.0001*
T+D	0.126 (0.016)	1	0.82	0.365
Season	-	2	86.55	<0.0001*
Temp:Season	-	2	1.56	0.212
RE:Season	-	2	5.77	0.0034*
Control	0.150 (0.018)	-	-	-
Inorganic N				
Control	0.228 (0.020)	-	-	-
Temperature	0.258 (0.027)	1	1.70	0.192
RE	0.248 (0.022)	1	25.44	<0.0001*
T+D	0.223 (0.021)	1	0.35	0.553
Season	-	2	145.76	<0.0001*
Temp:Season	-	2	0.823	0.440
RE:Season	-	2	2.43	0.090
T+D:Season	-	2	3.081	0.047*
Phosphate				
Control	0.0024 (0.0003)	-	-	-
Temperature	0.0025 (0.0003)	1	2.86	0.092
RE	0.0043 (0.0004)	1	19.58	<0.0001*
T+D	0.0034 (0.0004)	1	3.60	*0.058*
Season	-	2	41.94	<0.0001*
Temp:Season	-	2	0.364	0.695
RE:Season	-	2	11.23	<0.0001*

** Indicates a significant result*

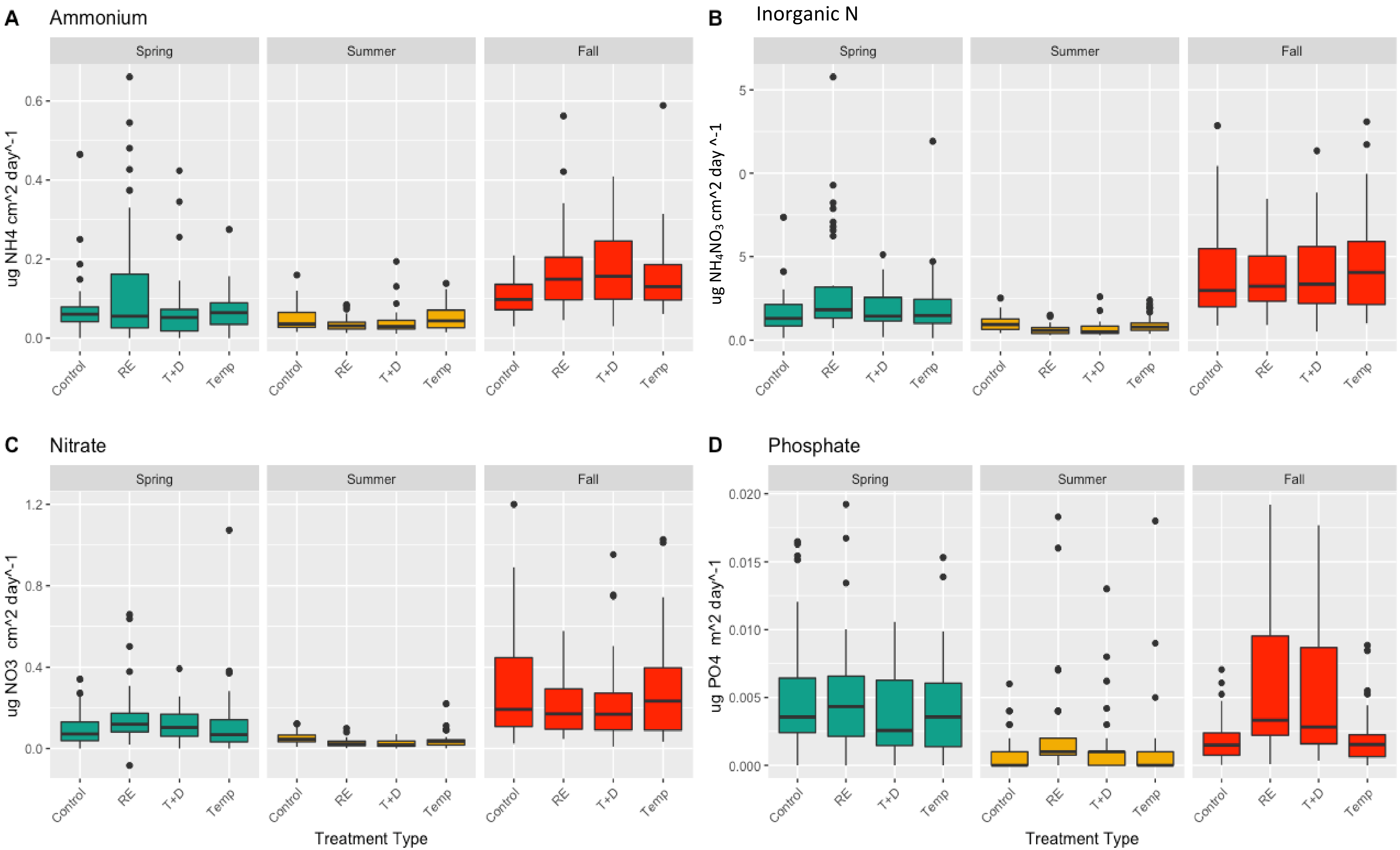

Figure 5: Average production rates of soil nutrients by treatment per season.

Table 4: Results of ANOVAs on GLS models of soil nutrient rates by treatment and season. Significant results are marked with '' and underlined. All means are rates in ug per cm^2 per day.*

	Spring			**Summer**			**Fall**		
	Mean	χ^2	p	Mean	χ^2	p	Mean	χ^2	p
Ammonium									
Control	0.074(0.01)	-	-	0.048(0.005)	-	-	0.106(0.006)	-	-
Temp	0.069(0.01)	2.52	0.11	0.052(0.005)	1.31	0.25	0.156(0.017)	5.43	0.02*
RE	0.124(0.03)	1.46	0.23	0.034(0.003)	3.80	0.05*	0.167(0.015)	5.52	0.02*
T+D	0.073(0.03)	1.50	0.22	0.040(0.006)	0.18	0.67	0.174(0.017)	2.80	0.09
Nitrate									
Control	0.089(0.01)	-	-	0.053(0.004)	-	-	0.305(0.04)	-	-
Temp	0.124(0.03)	0.092	0.76	0.041(0.006)	2.30	0.13	0.303(0.05)	0.040	0.84
RE	0.163(0.02)	2.75	0.10	0.027(0.002)	19.03	1.28e-5*	0.215(0.02)	2.94	0.09
T+D	0.114(0.01)	3.61	*0.06*	0.026(0.003)	1.84	0.17	0.238(0.04)	0.040	0.84
Inorganic N									
Control	0.166(0.02)	-	-	0.100(0.008)	-	-	0.412(0.04)	-	-
Temp	0.204(0.04)	1.40	0.24	0.092(0.009)	0.10	0.75	0.472(0.06)	1.32	0.25
RE	0.307(0.05)	4.10	0.04*	0.061(0.004)	17.0	3.8e-5*	0.382(0.03)	0.76	0.38
T+D	0.189(0.02)	5.12	0.02*	0.067(0.008)	1.20	0.27	0.412(0.04)	0.30	0.59
Phosphate									
Control	0.005(7.0e-4)	-	-	0.002(2.0e-4)	-	-	0.001(3.0e-4)	-	-
Temp	0.004(6.0e-4)	2.16	0.14	0.001(6.0e-4)	0.03	0.86	0.002(3.0e-4)	0.98	0.32
RE	0.005(6.0e-4)	0.01	0.92	0.002(6.0e-4)	2.31	0.13	0.006(7.0e-4)	13.8	2.07e-4*
T+D	0.004(5.0e-4)	0.04	0.84	0.002(4.0e-4)	2.42	0.12	0.005(7.0e-4)	2.23	0.14

3.2 Greenhouse gases fluxes

The net exchange of carbon dioxide (CO_2) and methane (CH_4) between the soil and the atmosphere was not significantly affected by any of the treatments. However, nitrous oxide (N_2O) net fluxes were significantly influenced by the temperature treatment (F=8.95, p=0.003), with a negative emission rate (net fixation) in the control plots, but a positive rate (net emission) in the temperature treatment plots (Table 5). There were no significant interactions between season and the treatments. However, all gases fluxes significantly varied between seasons (Table 5). Carbon dioxide emissions were significantly highest in spring and lowest in fall, with significant differences between all seasons (Figure 6A). Nitrous oxide was also found to be significantly different between all seasons (Figure 6B). Methane was fixated at the highest rate in spring (lowest rate of emission), with no significant differences between fall, winter, and summer. (Figure 6C).

Large variations in the response variable values inhibited our ability to detect statistically significant differences in GHG fluxes rates. However, observing seasonal averages yield insights

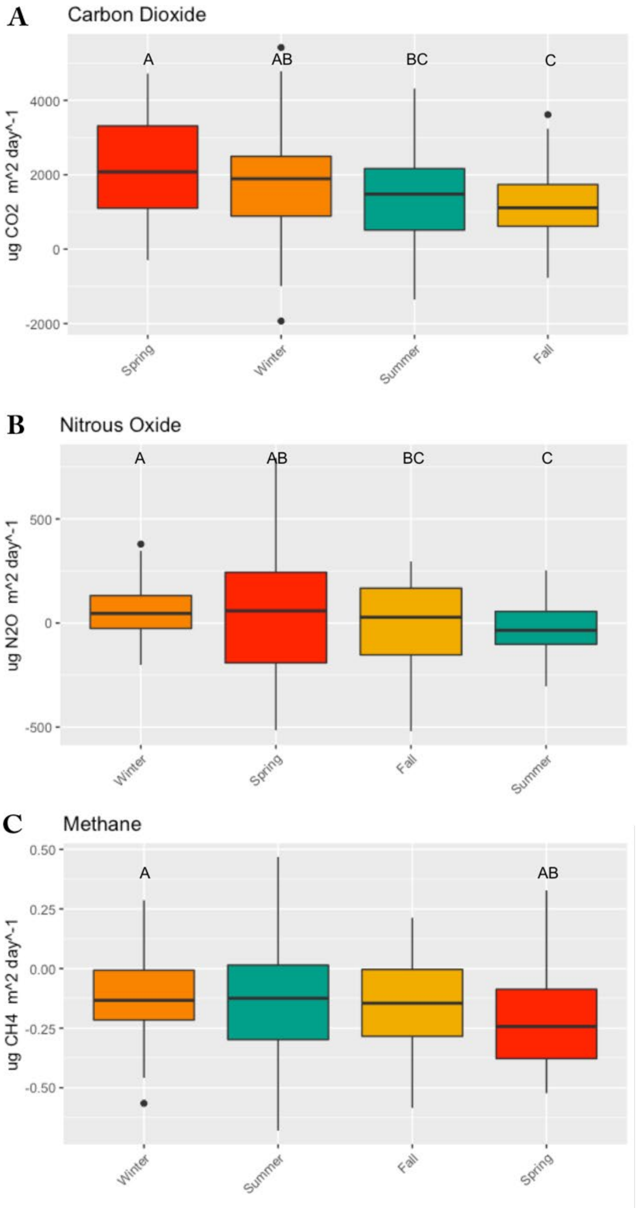

Figure 6: Average greenhouse gas fluxes per season in ug per m2/day. Letters representing significant differences between groups.

Table 5: Results of ANOVAs for each greenhouse gas. Means are reported in ug per m^2 per day. Standard errors of the means are included in brackets. Significant values are marked with ''*

	Mean	ANOVA		
Carbon dioxide		***D.F.***	**F**	***p***
Control	1781.5 (174.7)	-	-	-
Temperature	1437.1 (134.6)	1	2.39	0.123
RE	1772.0 (152.6)	1	0.54	0.469
T+D	1665.1 (145.9)	1	0.61	0.438
Season	-	3	9.99	3.25e-6*
Temp:Season	-	3	0.93	0.426
RE:Season	-	3	1.10	0.351
Temp:RE:Season	-	3	1.20	0.309
Nitrous oxide				
Control	-21.18 (27.5)	-	-	-
Temperature	27.27 (24.6)	1	8.95	0.003*
RE	46.65 (25.4)	1	0.56	0.454
T+D	15.29 (19.4)	1	1.87	0.174
Season	-	3	16.1	5.264e-9*
Temp:Season	-	3	1.38	0.251
RE:Season	-	3	0.274	0.844
Temp:RE:Season	-	3	0.758	0.519
Methane				
Control	-0.174 (0.025)	-	-	-
Temperature	-0.137 (0.025)	1	0.14	0.709
RE	-0.147 (0.026)	1	0.01	0.940
T+D	-0.168 (0.021)	1	1.76	0.186
Season	-	3	3.19	0.024*
Temp:Season	-	3	1.19	0.314
RE:Season	-	3	0.77	0.520
Temp:RE:Season	-	3	1.57	0.197

into how climate change stressors differentially impact GHG fluxes depending on the season (Table 6). Summer and fall CO_2 emissions were higher in all treatments than in control plots, while in spring and winter we observed the opposite trend, with higher rates in the control plots (Figure 7A).

Nitrous oxide fluxes were negatively impacted by temperature in spring (Figure 7B). Similarly, the rain exclusion treatment also reduced spring emissions by 67%, despite not being significantly different. Summer N_2O net fluxes rate were negative in the control plot, indicating net fixation under normal conditions. However, our temperature treatment reduced this net fixation by 49%. Furthermore, the rain exclusion treatment increased summer N_2O fluxes by 150%. As a result, the net rates were no longer negative, and the system became a net source of N_2O instead of a sink. In fall, the average flux in the control plots represented the greatest rate of fixation of any plot for the year. However, in the same season, the net N_2O flux became positive under the temperature, rain exclusion, and combination treatments, indicating a shift from fixation to source. Average winter N_2O fluxes were higher in the temperature and rain exclusion treatments than the control plots.

Lastly, the average CH_4 fluxes rates remained negative throughout the year under all treatments (Figure 7C). Control plots tended to show the highest rates of fixation in spring and lowest in summer. In fall, all plots were fixating around the same rate, while in winter the fixation rates under temperature (55%) and rain exclusion (34%) treatments were reduced, as compared to control plots.

Table 6: Results of ANOVAs on LMM models of greenhouse gases by treatment and season. Average rates of emission are expressed in ug per m^2 per day, with standard error in brackets. Significant results are marked with ''.*

	Spring			**Summer**			**Fall**			**Winter**		
	Mean	F	*p*	Mean	F	*p*	Mean	F	*p*	Mean	F	*p*
CO_2												
Control	2565.73(362.01)	-	-	1222.66(268.57)	-	-	973.15(263.26)	-	-	2349.72(346.81)	-	-
Temp	1742.80(203.35)	2.59	0.117	1392.98(250.72)	0.17	0.680	1084.53(249.58)	0.32	0.577	1540.12(340.81)	1.91	0.176
RE	2403.46(289.37)	0.34	0.565	1787.36(311.80)	0.82	0.369	1275.79(189.93)	1.80	0.189	1624.20(364.15)	0.98	0.330
T+D	2274.01(328.00)	1.37	0.250	1369.54(350.93)	0.97	0.329	1409.66(218.98)	0.001	0.975	1590.90(222.99)	1.61	0.212
N_2O												
Control	48.91(85.36)	-	-	-40.33(33.23)	-	-	-97.97(56.63)	-	-	13.31(27.35)	-	-
Temp	13.85(70.87)	8.86	0.013*	-19.81(21.70)	3.51	0.073	16.94(51.12)	0.22	0.649	92.86(33.87)	1.29	0.267
RE	16.30(78.22)	0.0004	0.984	25.93(35.40)	1.53	0.228	54.25(36.66)	0.02	0.904	89.80(37.08)	0.009	0.924
T+D	43.92(54.58)	0.33	0.576	-43.26(27.17)	0.05	0.820	31.34(39.76)	0.48	0.504	26.55(23.04)	3.87	0.060
CH_4												
Control	-0.253(0.050)	-	-	-0.102(0.049)	-	-	-0.198(0.057)	-	-	-0.146(0.037)	-	-
Temp	-0.224(0.052)	3.46	0.072	-0.140(0.051)	0.85	0.361	-0.111(0.042)	0.07	0.788	-0.066(0.048)	0.01	0.930
RE	-0.249(0.032)	0.93	0.343	-0.129(0.070)	0.54	0.466	-0.111(0.042)	0.05	0.828	-0.097(0.050)	0.38	0.541
T+D	-0.134(0.046)	1.13	0.295	-0.193(0.047)	0.06	0.813	-0.176(0.042)	3.2	0.081	-0.169(0.037)	3.12	0.086

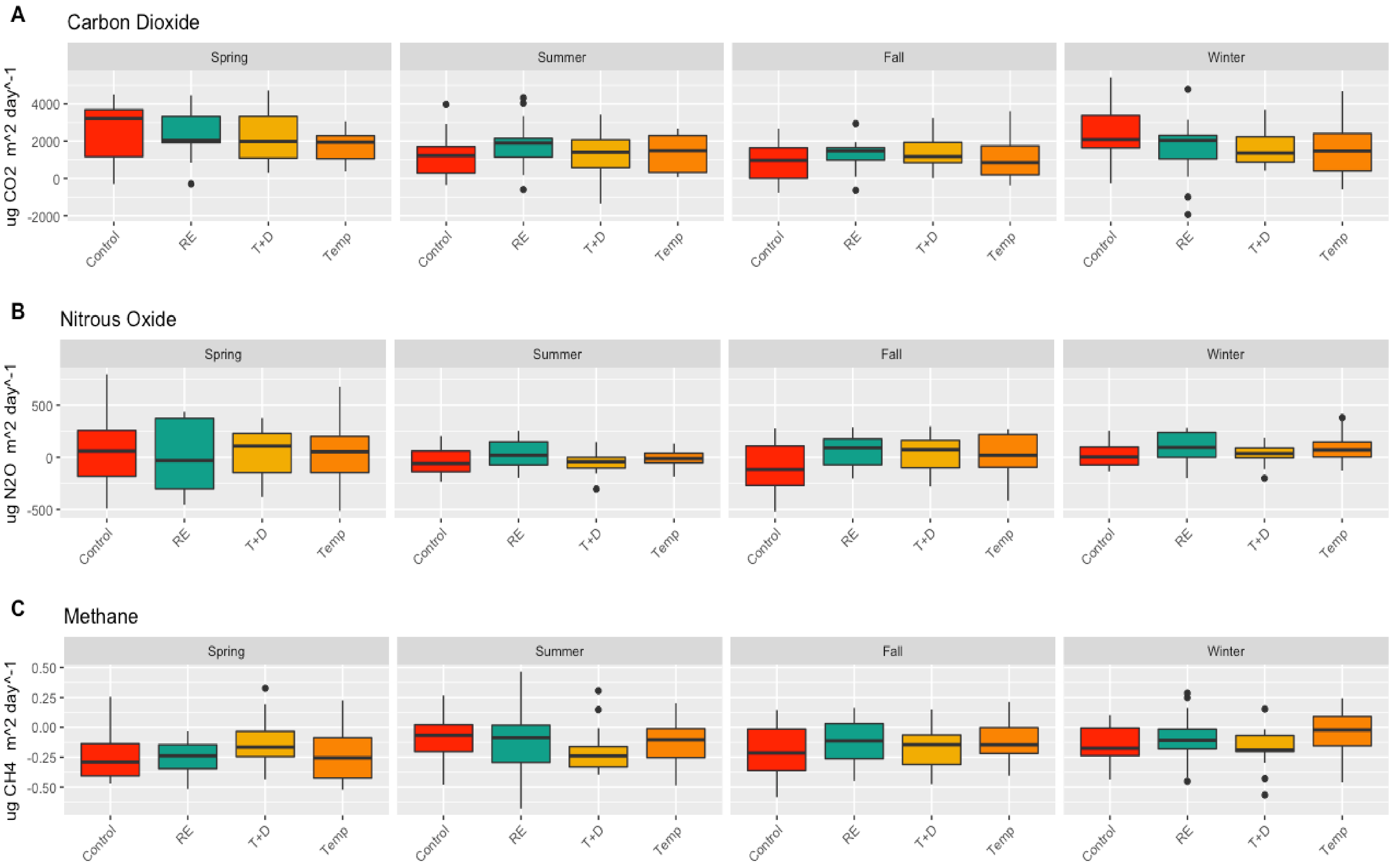

Figure 7: Average greenhouse gases fluxes by treatment in each sampling season.

4 Discussion

Los Alcornocales natural park is recognized as an ecologically, culturally, and economically valuable region and is considered and expected to be at risk under present and future climate change scenarios (Kim et al., 2017; Aronson et al., 2009). The impacts that these climatic changes will have on ecosystem functioning is difficult to forecast but of vital importance to investigate. The main aim of this study was to understand the impact of these projected changes in temperature and precipitation on important ecosystem processes, such as the rates of soil nutrient cycling and greenhouse gas fluxes. Our results demonstrate that these aforementioned alterations will result in significant changes in the availability of nitrogen and phosphorus, as well as in changes in the rate of exchange of greenhouse gases between the soil and the atmosphere, with significant increases in nitrous oxide rates, and potential decreases in sink capacity of methane, and increased emissions of carbon dioxide. Our results also provide valuable and novel information about the seasonal dynamics of these key ecosystem functions and how they will be impacted by future climate change.

4.1 Soil Nutrients Availability

Overall, soil ammonium, inorganic N, and phosphorus production significantly increased, while nitrate significantly decreased, under the rain exclusion treatment. Seasonal dynamics and the interaction with our treatments revealed more insights into how climate change may impact ecosystem functionality.

Spring is an important period for plant growth, thus changes in soil nutrient availability during this time can have important implications for forest functioning (Natalini et al., 2016; Liu, 2010). Inorganic nitrogen production was significantly increased under both rain exclusion and combination treatments, while nitrate was nearly significantly increased (p=0.06) under the combination treatment. It is likely that the increase in temperatures have caused a response in the microbial community under ideal seasonal growth conditions. However, the decrease in water availability due to the rain shelters and increased evapotranspiration (through the increase in temperatures) might have limited the capacity of plants to uptake those nutrients, leading to the observed accumulation of nutrients in the soil (Gessler et al, 2017; Homyak et al., 2017).

Summer in the south of the Iberian Peninsula is already a challenging season for both plant and soil microbial communities, with high temperatures and reduction in precipitation (Costa, 2018; Aronson et al., 2009). Our manipulative intensification in these environmental challenges resulted in a reduction in the availability of all the considered N pools. Our results support previous studies that suggested that intensified summer droughts can reduce nutrient production through alterations in the microbial communities involved in soil nutrient cycling (Bastida et al., 2019; Preece et al, 2019; Ren et al., 2017, 2018; Meisner et al., 2013; Hueso & Hernandez, 2012; Berard et al., 2012).

Interestingly, there was a remarkable increase in the phosphorus availability in fall under the rain exclusion treatment (as well as a similar but statistically non-significant increase under the combination treatment), together with an increase in soil ammonium production under both the rain exclusion and temperature treatments. During this sampling period, which occurred in November-December, there was a parallel increase in soil moisture after summer drought conditions (Figure 8), which likely triggered a recovery response in the microbial communities which took advantage of improving conditions and organic matter built up over the summer, resulting in a sharp release of available ammonium and phosphorus.

A response in the N cycle to drought conditions has also been documented by Homyak et al (2017), who found an increase in the soil availability of ammonium and stable levels of nitrate, and suggested the increase being due to the limited ability of plant uptake under drought conditions, and the limitation of nitrification under drought conditions to transform it. They also noted that, in drying soils, large fractions of the nitrified N can escape as gaseous N forms, which could at least partially help explain the decrease we found in nitrate in this study. Another possibility is discussed in a review by Booth et al. (2005), who suggested that decreases of nitrate in relation

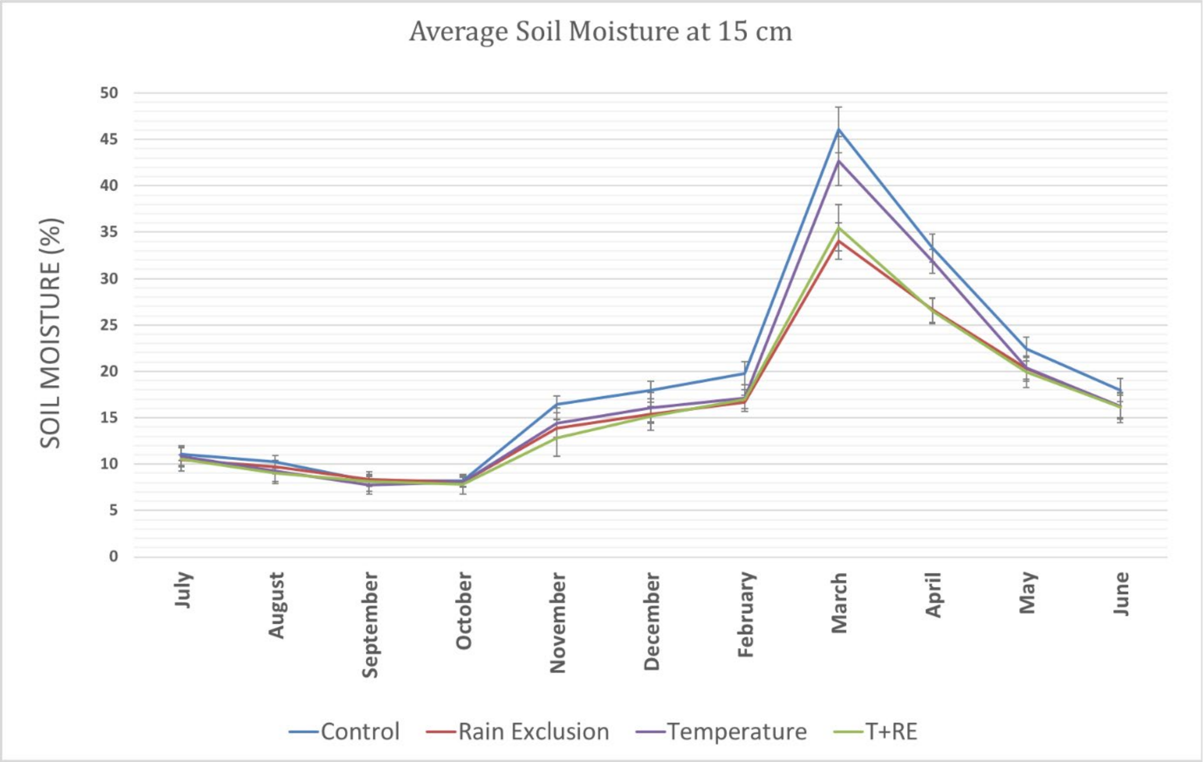

Figure 8: Average soil moisture percentage in the top 15cm of soil of the study site in each treatment over the sampling period.

to increasing amounts of ammonium might also be attributed to competition for space between mineralizing and nitrifying bacteria, as well as to interactions with soil C availability.

Another possible dynamic causing a release in soil nutrients is the so-called birch effect. Soil drying and rewetting events have also been shown to result in a release in C, N, and P as well as pulses of greenhouse gases such as CO_2 (Birch, 1958; Rodríguez et al., 2019; Morillas et al., 2017; Meisner et al 2015; Ouyang & Li, 2013; Jarvis et al, 2005). Thus, the increased availability of inorganic phosphorus and ammonium in fall also could also result from increased phosphorus and nitrogen mineralization processes due to drying-rewetting dynamics. Our field experiment supports previous lab-based studies that showed a release of inorganic phosphorus (Brodlin et al., 2019; Dihn et al. 2017; Bünemann et al. 2013; Butterly et al. 2011) and ammonium (Pezzola et al. 2019; Homyak et al. 2017) after experimental soil drying-wetting.

In summary, our results confirm that forecasted climate change-related alterations in temperature and precipitation (and its combination) might have a significant impact on the production of soil bioavailable nitrogen and phosphorus throughout the year, with noticeable releases in soil available ammonium, but likely with limited uptake by plant communities. These built-up soil nutrients, without a concomitant increase in the capacity of plants to uptake these nutrients (due to lower water availability), can result in significant losses to leaching or gaseous emissions and, on the long term, to the intensification of nutrient limitation of these already limited systems (Homyak et al., 2017; Matais et al 2010; Aronson et al., 2009). Such intensification can lead to stunted growth, and less resilience to further stressors such as prolonged or intensified droughts or pathogens such as *P. cinnamomi,* which is already severely impacting these forests (Costa, 2018; Kim et al., 2017), and even trigger a regime shift in vegetation (Rodriguez et al., 2019; Liu et al., 2018). Together, our results clearly show that soil nutrient cycling processes will not stay the same during more extreme or more frequent future droughts and increases in temperature, which alone and combined can stimulate excessive releases of soil nutrients which can be lost as leachates or gaseous emissions. This confirms that forecasted changes in temperature and precipitation will change key soil processes and put ecosystem functioning at risk for reduced resilience against continuous or additional stressors, such as further climate changes.

4.2 Greenhouse Gases Fluxes

The exchange of greenhouse gases between the soil and the atmosphere were not shown to be as sensitive to changes in temperature and rainfall as soil nutrients transformation rates, with only nitrous oxide showing a significant increase in response to our temperature treatment, particularly in spring. Our inability to detect statistically significant effects for methane and carbon dioxide is likely due to large variations in the response variable values. However, we can still evaluate some ecologically-relevant trends and differences among seasons.

Nitrous oxide can be produced by both denitrification and nitrification processes, depending on soil water content, and is stimulated by higher temperatures (IPNI, 2019; Álvaro-Fuentes et al., 2017; Homyak et al., 2017). Thus, the increases of N_2O emissions in fall and winter can be due to similar reasons as the increase in ammonium and nitrate in spring –the microbial community was stimulated by improving conditions with increased rainfall, and particularly higher temperatures under the temperature treatments. Additionally, emissions of nitrous oxide have also been shown to interact with increases in soil mineral N and C availability (Van Groenigen et al., 2015; Luo et al., 2013). Thus, as N_2O is mainly produced through heterotrophic nitrification in aerobic soils using N from ammonium pools (Butterbach-Bahl et al., 2013), it's possible that the increase in the soil ammonium availability also bolstered the increase in N_2O, particularly under the lower moisture conditions and higher temperatures of our treatments (Van Groenigen et al., 2015). In any case, our results clearly show that the forecasted changes in precipitation and temperature due to climate change can limit the capacity of these systems to uptake atmospheric nitrous oxide, and even transform the system into a net source of this powerful greenhouse gas, representing a paradigmatic example of positive biotic feedback of climate change (Durán et al. 2013).

As expected, our results confirm that these forest function as net sinks of atmospheric methane. However, in general, the amount of methane fixed by these soils was particularly high in the control plots. Our results suggest that our treatments are able to produce significant decreases in methane fixation rates throughout the year. This can be indicative of challenging conditions to methanotrophic bacterial communities (Luo et al., 2013; Ho & Frenzel, 2012). Previous studies showed a strong short-term resilience for methanotrophs under drought conditions (Ho et al., 2016). However, in the long-term, or with more extreme weather events, the structure and function of this bacterial community may be altered (Bodelier & Steenbergh, 2014; Ho & Frenzel, 2012; Mohanty et al., 2007). Our results shed some light on the potential effects of climate change on methanotrophic community. Thus, although a high temporal and spatial variability limited our capacity to consistently find significant effects, the strong and consistent observed trends suggest that under natural conditions continued increases in soil temperature and decreases in soil moisture can indeed alter the capacity of Mediterranean forests to uptake methane, at least in some seasons.

Carbon dioxide fluxes typically increase in response to variations in temperature and rainfall (Canarini et al., 2017; Smith et al., 2018; Oertel et al., 2016; Leifield, 2007). However, in our study, we did not detect this kind of response. This can be due to a few reasons. First, most studies focusing on the effects of climate change on soil respiration (i.e. soil CO_2 emission) have been carried out under controlled, and sometimes drastic lab conditions. On the contrary, our experiment was carried out under field conditions and with realistic changes in soil temperature and precipitation, so the effects in soil respiration may be more subtle or may even take more time to arise. Also, the microbial community in this region is likely to be adapted to cope with subtle and somehow transient (one year) changes in temperature and soil moisture but might still suffer in response to longer-term stressors or more extreme climatic events (Flores-Rentería et al., 2018; Curiel-Yuste et al., 2014). Indeed, this study was conducted only a year or two after the initial setup of the experiment and is only capturing the dynamics of one full year, so further monitoring of the study site may reveal longer-term changes. Likewise, all of soil nutrients and greenhouse gases could have been affected by these reasons as well. Thus, this study highlights that longer monitoring times of climate change field treatments are important to uncover longer-term trends and to account for intra and inter-annual variations.

5 Conclusion

Soil nutrient cycle integrity is important in these already nutrient-limited systems, and as this field study has shown, forecasted increases in temperature and rainfall reduction (and the combination of both) are likely to significantly impact soil nutrient cycles and greenhouse gases emissions. This study has shown that reductions in rainfall particularly impact soil nutrient availability, and possibly causes a decrease in nutrient uptake in plants leading to an accumulation of nutrients in the soil, increasing the risk of nutrient loss by leaching or gaseous emissions. Indeed, this study also demonstrate that increases in temperature and decreases in precipitation might also increase soil nitrous oxide emissions and likely decrease the capacity of this ecosystem to uptake methane from the atmosphere, which could contribute to further global warming.

This valuable cork-oak ecosystem is already under the strain of multiple stressors and understanding future challenges will better prepare managements strategies to mitigate these changes, and to understand what processes will be most damaged by climate change. Our study clearly demonstrates the capacity of climate change to alter soil functioning in these ecosystems. However, further studies on how this study site continues to react to these treatments on the long-term, how tree growth and vegetation dynamics are affected by the treatments, and an exhaustive analysis on the how the microbial communities are changing would be highly valuable and recommendable.

6 References

Álvaro-Fuentes, J., Arrúe, J. L., Bielsa, A., Cantero-Martínez, C., Plaza-Bonilla, D., & Paustian, K. (2017). Simulating climate change and land use effects on soil nitrous oxide emissions in Mediterranean conditions using the Daycent model. *Agriculture, Ecosystems and Environment*, *238*, 78–88. https://doi.org/10.1016/j.agee.2016.07.017

Aponte, C., Matías, L., González-Rodríguez, V., Castro, J., García, L. V., Villar, R., & Marañón, T. (2014). Soil nutrients and microbial biomass in three contrasting Mediterranean forests. *Plant and Soil*, *380*(1), 57–72. https://doi.org/10.1007/s11104-014-2061-5

Aronson, J. (2009). *Cork Oak Woodlands on the Edge: Ecology, Adaptive Management, and Restoration* (James Aronson, J. S. Pereira, & J. U. G. Pausas, eds.). Retrieved from https://www.uv.es/jgpausas/corkoak/cork-oak-book.pdf

Avila, J. M., Linares, J. C., García-Nogales, A., Sánchez, M. E., & Gómez-Aparicio, L. (2017). Across-scale patterning of plant–soil–pathogen interactions in Quercus suber decline. *European Journal of Forest Research*, *136*(4), 677–688. https://doi.org/10.1007/s10342-017-1064-1

Avila, José M., Gallardo, A., Ibáñez, B., & Gómez-Aparicio, L. (2016). Quercus suber dieback alters soil respiration and nutrient availability in Mediterranean forests. *Journal of Ecology*, *104*(5), 1441–1452. https://doi.org/10.1111/1365-2745.12618

Avila, José Manuel, Gallardo, A., & Gómez-Aparicio, L. (2019). Pathogen-induced tree mortality interacts with predicted climate change to alter soil respiration and nutrient availability in Mediterranean systems. *Biogeochemistry*, *142*(1), 53–71. https://doi.org/10.1007/s10533-018-0521-3

Bardgett, Richard D.; van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*, 505–511.

Bardgett, R. D., & van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*(7528), 505–511. https://doi.org/10.1038/nature13855

Bastida, F., López-Mondéjar, R., Baldrian, P., Andrés-Abellán, M., Jehmlich, N., Torres, I. F., ... López-Serrano, F. R. (2019). When drought meets forest management: Effects on the soil microbial community of a Holm oak forest ecosystem. *Science of the Total Environment*, *662*, 276–286. https://doi.org/10.1016/j.scitotenv.2019.01.233

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A. L., & Gros, R. (2011). Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. *European Journal of Soil Biology*, *47*(6), 333–342. https://doi.org/10.1016/j.ejsobi.2011.08.004

Bernhard, A. (2010). The Nitrogen Cycle: Processes, Players, and Human Impact. Retrieved August 14, 2019, from Nature Education Knowledge website: https://www.nature.com/scitable/knowledge/library/the-nitrogen-cycle-processes-players-and-human-15644632/#

Birch, H. F. (1958). The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil*, *10*(1), 9–31. https://doi.org/10.1007/BF01343734

Bodelier, P. L. E., & Steenbergh, A. K. (2014). Interactions between methane and the nitrogen cycle in light of climate change. *Current Opinion in Environmental Sustainability*, *9–10*, 26–36. https://doi.org/10.1016/j.cosust.2014.07.004

Bokhorst, S., Huiskes, A., Aerts, R., Convey, P., Cooper, E. J., Dalen, L., ... Dorrepaal, E. (2013). Variable temperature effects of Open Top Chambers at polar and alpine sites explained by irradiance and snow depth. *Global Change Biology*. https://doi.org/10.1111/gcb.12028

Brödlin, D., Kaiser, K., Kessler, A., & Hagedorn, F. (2019). Drying and rewetting foster phosphorus depletion of forest soils. *Soil Biology and Biochemistry*, *128*(September 2018), 22–34. https://doi.org/10.1016/j.soilbio.2018.10.001

Bünemann, E. K., Keller, B., Hoop, D., Jud, K., Boivin, P., & Frossard, E. (2013). Increased availability of phosphorus after drying and rewetting of a grassland soil: processes and plant use. *Plant and Soil*, *370*(1–2), 511–526. https://doi.org/10.1007/s11104-013-1651-y

Butterly, C. R., McNeill, A. M., Baldock, J. A., & Marschner, P. (2011). Rapid changes in carbon and phosphorus after rewetting of dry soil. *Biology and Fertility of Soils*, *47*(1), 41–50. https://doi.org/10.1007/s00374-010-0500-x

Canarini, A., Kiær, L. P., & Dijkstra, F. A. (2017). Soil carbon loss regulated by drought intensity and available substrate: A meta-analysis. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2017.04.020

Carnicer, J., Domingo-Marimon, C., Ninyerola, M., Camarero, J. J., Bastos, A., López-Parages, J., ... Pons, X. (2019). Regime shifts of Mediterranean forest carbon uptake and reduced resilience driven by multidecadal ocean surface temperatures. *Global Change Biology*, gcb.14664. https://doi.org/10.1111/gcb.14664

Chapin, F. S., McFarland, J., David McGuire, A., Euskirchen, E. S., Ruess, R. W., & Kielland, K. (2009). The changing global carbon cycle: Linking plant-soil carbon dynamics to global consequences. *Journal of Ecology*, *97*(5), 840–850. https://doi.org/10.1111/j.1365-2745.2009.01529.x

Corcobado, T., Cubera, E., Juárez, E., Moreno, G., & Solla, A. (2014). Drought events determine performance of Quercus ilex seedlings and increase their susceptibility to Phytophthora cinnamomi. *Agricultural and Forest Meteorology*. https://doi.org/10.1016/j.agrformet.2014.02.007

Costa, A., Barbosa, I., Roussado, C., Graça, J., & Spiecker, H. (2016). Climate response of cork growth in the Mediterranean oak (Quercus suber L.) woodlands of southwestern Portugal. *Dendrochronologia*, *38*, 72–81. https://doi.org/10.1016/j.dendro.2016.03.007

Costa, Augusta. (2016). *"Effects of climate change on cork oak woodlands"*.

Costa, Augusta, Madeira, M., Lima Santos, J., & Oliveira, Â. (2011). Change and dynamics in Mediterranean evergreen oak woodlands landscapes of Southwestern Iberian Peninsula. *Landscape and Urban Planning*, *102*(3), 164–176. https://doi.org/10.1016/j.landurbplan.2011.04.002

Courtney Johnson, Greg Albrecht, Quirine Ketterings, Jen Beckman, & Kristen Stockin. (2005). Nitrogen Basics-The Nitrogen Cycle Agronomy Fact Sheet Series. *Cornell University Cooperative Extension*, 1–2. Retrieved from http://cceonondaga.org/resources/nitrogen-basics-the-nitrogen-cycle

de Dato, G. D., De Angelis, P., Sirca, C., & Beier, C. (2010). Impact of drought and increasing temperatures on soil CO2 emissions in a Mediterranean shrubland (gariga). *Plant and Soil*, *327*(1–2), 153–166. https://doi.org/10.1007/s11104-009-0041-y

Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., ... Singh, B. K. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, *7*, 1–8. https://doi.org/10.1038/ncomms10541

Díaz, M. A., Bown, H. E., Fuentes, J. P., & Martínez, A. M. (2018). Soils act as sinks or sources of CH4 depending on air-filled porosity in sclerophyllous ecosystems in semiarid central Chile. *Applied Soil Ecology*, *130*(February), 13–20. https://doi.org/10.1016/j.apsoil.2018.05.017

Dinh, M. Van, Guhr, A., Spohn, M., & Matzner, E. (2017). Release of phosphorus from soil bacterial and fungal biomass following drying/rewetting. *Soil Biology and Biochemistry*, *110*, 1–7. https://doi.org/10.1016/j.soilbio.2017.02.014

Duan, P., Wu, Z., Zhang, Q., Fan, C., & Xiong, Z. (2018). Thermodynamic responses of ammonia-oxidizing archaea and bacteria explain N2O production from greenhouse vegetable soils. *Soil Biology and Biochemistry*, *120*(January), 37–47. https://doi.org/10.1016/j.soilbio.2018.01.027

Duque-Lazo, J., Navarro-Cerrillo, R. M., & Ruíz-Gómez, F. J. (2018). Assessment of the future stability of cork oak (Quercus suber L.) afforestation under climate change scenarios in Southwest Spain. *Forest Ecology and Management*. https://doi.org/10.1016/j.foreco.2017.11.042

Durán, J., Delgado-Baquerizo, M., Rodríguez, A., Covelo, F., & Gallardo, A. (2013). Ionic exchange membranes (IEMs): A good indicator of soil inorganic N production. *Soil Biology and Biochemistry*, *57*, 964–968. https://doi.org/10.1016/j.soilbio.2012.07.016

Durán, Jorge, Delgado-Baquerizo, M., Dougill, A. J., Guuroh, R. T., Linstädter, A., Thomas, A. D., ... Delgado-Baquerizo, M. (2018). Temperature and aridity regulate spatial variability of soil multifunctionality in drylands across the globe. *Ecology*, *0*(0), 1–10. https://doi.org/10.1002/ecy.2199

European Commission. (2009). *Natura 2000 in the Mediterranean Region*.

https://doi.org/10.2779/77695

Flores-Rentería, D., Curiel Yuste, J., Valladares, F., & Rincón, A. (2018). Soil legacies determine the resistance of an experimental plant-soil system to drought. *Catena*, *166*(November 2017), 271–278. https://doi.org/10.1016/j.catena.2018.04.011

Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis, S., ... Voss, M. (2013). The global nitrogen cycle in the Twentyfirst century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130164. https://doi.org/10.1098/rstb.2013.0164

Gauquelin, T., Michon, G., Joffre, R., Duponnois, R., Génin, D., Fady, B., ... Baldy, V. (2018). Mediterranean forests, land use and climate change: a social-ecological perspective. *Regional Environmental Change*, *18*(3), 623–636. https://doi.org/10.1007/s10113-016-0994-3

Gessler, A., Schaub, M., & Mcdowell, N. G. (2017). The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist*, (214), 513–520. https://doi.org/10.1111/nph.14340

Gómez-Aparicio, L., Domínguez-Begines, J., Kardol, P., Ávila, J. M., Ibáñez, B., & García, L. V. (2017). Plant-soil feedbacks in declining forests: implications for species coexistence. *Ecology*, *98*(7), 1908–1921. https://doi.org/10.1002/ecy.1864

Göransson, H., Godbold, D. L., Jones, D. L., & Rousk, J. (2013). Bacterial growth and respiration responses upon rewetting dry forest soils: Impact of drought-legacy. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2012.08.031

GRDC. (2014). *Denitrification: bacteria convert soil nitrate to gases*. Retrieved from www.grdc.com.au

Grillakis, M. G. (2019). Increase in severe and extreme soil moisture droughts for Europe under climate change. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2019.01.001

Guillot, E., Hinsinger, P., Dufour, L., Roy, J., & Bertrand, I. (2019). With or without trees: Resistance and resilience of soil microbial communities to drought and heat stress in a Mediterranean agroforestry system. *Soil Biology and Biochemistry*, *129*(June 2018), 122–135. https://doi.org/10.1016/j.soilbio.2018.11.011

Hansen, C. B. (2007). Generalized least squares inference in panel and multilevel models with serial correlation and fixed effects. *Journal of Econometrics*, *140*(2), 670–694. https://doi.org/10.1016/J.JECONOM.2006.07.011

Harrison, X., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D., Goodwin, C., ... Inger, R. (2018). Introduction to mixed effects models. *A Beginner's Guide to GLM and GLMM with R*, 116–132. https://doi.org/10.7717/peerj.4794

Hinojosa, M. B., Parra, A., Ramírez, D. A., Carreira, J. A., García-Ruiz, R., & Moreno, J. M. (2012). Effects of drought on soil phosphorus availability and fluxes in a burned Mediterranean shrubland. *Geoderma*, *191*, 61–69. https://doi.org/10.1016/j.geoderma.2012.01.015

Ho, A., & Frenzel, P. (2012). Heat stress and methane-oxidizing bacteria: Effects on activity and population dynamics. *Soil Biology and Biochemistry*, *50*, 22–25. https://doi.org/10.1016/j.soilbio.2012.02.023

Homet, P., González, M., Matías, L., Godoy, O., Pérez-Ramos, I. M., García, L. V., & Gómez-Aparicio, L. (2019). Exploring interactive effects of climate change and exotic pathogens on Quercus suber performance: Damage caused by Phytophthora cinnamomi varies across contrasting scenarios of soil moisture. *Agricultural and Forest Meteorology*, *276–277*, 107605. https://doi.org/10.1016/J.AGRFORMET.2019.06.004

Homyak, P. M., Allison, S. D., Huxman, T. E., Goulden, M. L., & Treseder, K. K. (2017). Effects of Drought Manipulation on Soil Nitrogen Cycling: A Meta-Analysis. *Journal of Geophysical Research: Biogeosciences*, *122*(12), 3260–3272. https://doi.org/10.1002/2017JG004146

Hu, H. W., Macdonald, C. A., Trivedi, P., Anderson, I. C., Zheng, Y., Holmes, B., ... Singh, B. K. (2016). Effects of climate warming and elevated CO 2 on autotrophic nitrification and nitrifiers in dryland ecosystems. *Soil Biology and Biochemistry*, *92*, 1–15. https://doi.org/10.1016/j.soilbio.2015.09.008

Hueso, S., García, C., & Hernández, T. (2012). Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2012.03.026

Hyland, C., Ketterings, Q., Dewing, D., Stockin, K., Czymmek, K., Albrecht, G., & Geohring, L. (2005).

Phosphorous factsheet. *Cornell University Cooperative Extension*, http://ktisis.cut.ac.cy/?locale=en.

IPNI. (n.d.-a). *Nitrification: How Fast?* Retrieved from http://www.ipni.net/publication/nitrogen-en.nsf/0/7F7F448C4D064A5985257C13004C83A3/$FILE/NitrogenNotes-EN-04.pdf

IPNI. (n.d.-b). *Nitrogen denitrification.* (3). Retrieved from www.ipni.net/publications

IUCN. (n.d.). Mediterranean-Type Ecosystems | IUCN. Retrieved August 8, 2019, from 2019 website: https://www.iucn.org/commissions/commission-ecosystem-management/our-work/cems-specialist-groups/mediterranean-type-ecosystems

Jarvis, P., Rey, A., Petsikos, C., Rayment, M., Pereira, J., David, J., ... Valentini, R. (2005). *Drying and wetting soils stimulates decomposition and carbon dioxide emission: the "Birch Effect". (Unpublished).* (September 2004), 929–940.

Jiménez-Chacón, A., Homet, P., Matías, L., Gómez-Aparicio, L., & Godoy, O. (2018). Fine scale determinants of soil litter fauna on a mediterranean mixed oak forest invaded by the exotic soil-borne pathogen Phytophthora cinnamomi. *Forests*, *9*(4), 1–16. https://doi.org/10.3390/f9040218

Kim, H. N., Jin, H. Y., Kwak, M. J., Khaine, I., You, H. N., Lee, T. Y., ... Woo, S. Y. (2017). Why does Quercus suber species decline in Mediterranean areas? *Journal of Asia-Pacific Biodiversity*, *10*(3), 337–341. https://doi.org/10.1016/j.japb.2017.05.004

Kool, D. M., Dolfing, J., Wrage, N., & Van Groenigen, J. W. (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biology and Biochemistry*, *43*(1), 174–178. https://doi.org/10.1016/j.soilbio.2010.09.030

Koutroulis, A. G. (2019). Dryland changes under different levels of global warming. *Science of The Total Environment*, *655*, 482–511. https://doi.org/10.1016/J.SCITOTENV.2018.11.215

Lagacherie, P., Álvaro-Fuentes, J., Annabi, M., Bernoux, M., Bouarfa, S., Douaoui, A., ... Raclot, D. (2018). Managing Mediterranean soil resources under global change: expected trends and mitigation strategies. *Regional Environmental Change*, *18*(3), 663–675. https://doi.org/10.1007/s10113-017-1239-9

Lei, X. (2010). *Assessment of Climate Change Impacts on Cork Oak in Western Mediterranean Regions: A Comparative Analysis of Extreme Indices.* Retrieved from https://pdfs.semanticscholar.org/93db/4de3521ae445843b83d46860cabd4c3942cd.pdf

Leifeld, J. (2007). Soils as sources and sinks of greenhouse gases. *Geological Society, London, Special Publications*, *266*(1), 23–44. https://doi.org/10.1144/gsl.sp.2006.266.01.03

Leitner, S., Homyak, P. M., Blankinship, J. C., Eberwein, J., Jenerette, G. D., Zechmeister-Boltenstern, S., & Schimel, J. P. (2017). Linking NO and N 2 O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils. *Soil Biology and Biochemistry*, *115*, 461–466. https://doi.org/10.1016/j.soilbio.2017.09.005

Li, J. T., Wang, J. J., Zeng, D. H., Zhao, S. Y., Huang, W. L., Sun, X. K., & Hu, Y. L. (2018). The influence of drought intensity on soil respiration during and after multiple drying-rewetting cycles. *Soil Biology and Biochemistry*, *127*(May), 82–89. https://doi.org/10.1016/j.soilbio.2018.09.018

Li, Y., Chapman, S. J., Nicol, G. W., & Yao, H. (2018). Nitrification and nitrifiers in acidic soils. *Soil Biology and Biochemistry*, *116*(October 2017), 290–301. https://doi.org/10.1016/j.soilbio.2017.10.023

Liu, D., Ogaya, R., Barbeta, A., Yang, X., & Peñuelas, J. (2018). Long-term experimental drought combined with natural extremes accelerate vegetation shift in a Mediterranean holm oak forest. *Environmental and Experimental Botany*, *151*(January), 1–11. https://doi.org/10.1016/j.envexpbot.2018.02.008

Luo, G. J., Kiese, R., Wolf, B., & Butterbach-Bahl, K. (2013). Effects of soil temperature and moisture on methane uptake and nitrous oxide emissions across three different ecosystem types. *Biogeosciences*, *10*(5), 3205–3219. https://doi.org/10.5194/bg-10-3205-2013

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I. A., Ciais, P., ... Peñuelas, J. (2017). Global patterns of phosphatase activity in natural soils. *Scientific Reports*, *7*(1), 1–13. https://doi.org/10.1038/s41598-017-01418-8

Mariotti, A., Pan, Y., Zeng, N., & Alessandri, A. (2015). Long-term climate change in the Mediterranean region in the midst of decadal variability. *Climate Dynamics*, *44*(5–6), 1437–1456.

https://doi.org/10.1007/s00382-015-2487-3

Martins, C. S. C., Macdonald, C. A., Anderson, I. C., & Singh, B. K. (2016). Feedback responses of soil greenhouse gas emissions to climate change are modulated by soil characteristics in dryland ecosystems. *Soil Biology and Biochemistry*, *100*, 21–32. https://doi.org/10.1016/j.soilbio.2016.05.007

Martins, C. S. C., Nazaries, L., Macdonald, C. A., Anderson, I. C., & Singh, B. K. (2015). Water availability and abundance of microbial groups are key determinants of greenhouse gas fluxes in a dryland forest ecosystem. *Soil Biology and Biochemistry*, *86*, 5–16. https://doi.org/10.1016/j.soilbio.2015.03.012

Matesanz, S., & Valladares, F. (2014). Ecological and evolutionary responses of Mediterranean plants to global change. *Environmental and Experimental Botany*, *103*, 53–67. https://doi.org/10.1016/j.envexpbot.2013.09.004

MATÍAS, L., CASTRO, J., & ZAMORA, R. (2011). Soil-nutrient availability under a global-change scenario in a Mediterranean mountain ecosystem. *Global Change Biology*, *17*(4), 1646–1657. https://doi.org/10.1111/j.1365-2486.2010.02338.x

Meisner, A., Bååth, E., & Rousk, J. (2013). Microbial growth responses upon rewetting soil dried for four days or one year. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2013.07.014

Meisner, A., Rousk, J., & Bååth, E. (2015). Prolonged drought changes the bacterial growth response to rewetting. *Soil Biology and Biochemistry*, *88*, 314–322. https://doi.org/10.1016/j.soilbio.2015.06.002

Menzies, N. (2009). *THE SCIENCE OF PHOSPHORUS NUTRITION: FORMS IN THE SOIL, PLANT UPTAKE, AND PLANT RESPONSE. - Grains Research and Development Corporation*. Retrieved from Grains Research and Development Corporation website: https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2009/02/the-science-of-phosphorus-nutrition-forms-in-the-soil-plant-uptake-and-plant-response

Mohanty, S. R., Bodelier, P. L. E., & Conrad, R. (2007). Effect of temperature on composition of the methanotrophic community in rice field and forest soil. *FEMS Microbiology Ecology*, *62*(1), 24–31. https://doi.org/10.1111/j.1574-6941.2007.00370.x

Moricca, S., Linaldeddu, B. T., Ginetti, B., Scanu, B., Franceschini, A., & Ragazzi, A. (2016). Endemic and Emerging Pathogens Threatening Cork Oak Trees: Management Options for Conserving a Unique Forest Ecosystem. *Plant Disease*, *100*(11), 2184–2193. https://doi.org/10.1094/PDIS-03-16-0408-FE

Morillas, L., Durán, J., Rodríguez, A., Roales, J., Gallardo, A., Lovett, G. M., & Groffman, P. M. (2015). Nitrogen supply modulates the effect of changes in drying-rewetting frequency on soil C and N cycling and greenhouse gas exchange. *Global Change Biology*, *21*(10), 3854–3863. https://doi.org/10.1111/gcb.12956

Morillas, L., Roales, J., Portillo-Estrada, M., & Gallardo, A. (2017). Wetting-drying cycles influence on soil respiration in two Mediterranean ecosystems. *European Journal of Soil Biology*, *82*, 10–16. https://doi.org/10.1016/j.ejsobi.2017.07.002

Myhre, G., Shindell, D., F.-M. Bréon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. M., & T. Nakajima, A. Robock, G. Stephens, T. T. and H. Z. (2013). *Anthropogenic and Natural Radiative Forcing. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Retrieved from https://www.ipcc.ch/site/assets/uploads/2018/02/WG1AR5_Chapter08_FINAL.pdf

Natalini, F., Alejano, R., Vázquez-Piqué, J., Cañellas, I., & Gea-Izquierdo, G. (2016). The role of climate change in the widespread mortality of holm oak in open woodlands of Southwestern Spain. *Dendrochronologia*, *38*, 51–60. https://doi.org/10.1016/j.dendro.2016.03.003

Naylor, D., & Coleman-Derr, D. (2018). Drought Stress and Root-Associated Bacterial Communities. *Frontiers in Plant Science*, *8*(January), 1–16. https://doi.org/10.3389/fpls.2017.02223

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., & Erasmi, S. (2016). Greenhouse gas emissions from soils—A review. *Chemie Der Erde - Geochemistry*, *76*(3), 327–352.

https://doi.org/10.1016/j.chemer.2016.04.002

Ouyang, Y., & Li, X. (2013). Recent research progress on soil microbial responses to drying–rewetting cycles. *Acta Ecologica Sinica*, *33*(1), 1–6. https://doi.org/10.1016/j.chnaes.2012.12.001

Ozturk, T., Ceber, Z. P., Türkeş, M., & Kurnaz, M. L. (2015). Projections of climate change in the Mediterranean Basin by using downscaled global climate model outputs. *International Journal of Climatology*, *35*(14), 4276–4292. https://doi.org/10.1002/joc.4285

Peñuelas, J., Sardans, J., Filella, I., Estiarte, M., Llusià, J., Ogaya, R., ... Terradas, J. (2018). Assessment of the impacts of climate change on Mediterranean terrestrial ecosystems based on data from field experiments and long-term monitored field gradients in Catalonia. *Environmental and Experimental Botany*, *152*(December 2016), 49–59. https://doi.org/10.1016/j.envexpbot.2017.05.012

Pessarakli, M. (1999). *Handbook of plant and crop stress*. M. Dekker.

Pezzolla, D., Cardenas, L. M., Mian, I. A., Carswell, A., Donovan, N., Dhanoa, M. S., & Blackwell, M. S. A. (2019). Responses of carbon, nitrogen and phosphorus to two consecutive drying-rewetting cycles in soils. *Journal of Plant Nutrition and Soil Science*, *182*(2), 217–228. https://doi.org/10.1002/jpln.201800082

Poblador, S., Lupon, A., Sabaté, S., & Sabater, F. (2017). Soil water content drives spatiotemporal patterns of CO2 and N2O emissions from a Mediterranean riparian forest soil. *Biogeosciences*, *14*(18), 4195–4208. https://doi.org/10.5194/bg-14-4195-2017

Pold, G., & DeAngelis, K. (2013). Up Against The Wall: The Effects of Climate Warming on Soil Microbial Diversity and The Potential for Feedbacks to The Carbon Cycle. *Diversity*, *5*(2), 409–425. https://doi.org/10.3390/d5020409

Preece, C., Verbruggen, E., Liu, L., Weedon, J. T., & Peñuelas, J. (2019). Effects of past and current drought on the composition and diversity of soil microbial communities. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2018.12.022

Pressarakli, M. (1999). *Handbook of Plant and Crop Stress* (2d Ed.). Retrieved from https://books.google.de/books?id=xsobnlXZBwQC&pg=PA272&lpg=PA272&dq=Since+ion+diffusion+to+the+root+is+very+often+the+step+limiting+nutrient+uptake,+a+decrease+in+soil+water+availability+can+affect+plant+growth&source=bl&ots=xdl3ER5NAI&sig=ACfU3U30h0glyjx

Protected Planet. (n.d.). Los Alcornocales | Protected Planet. Retrieved August 17, 2019, from 201 website: https://www.protectedplanet.net/los-alcornocales-natural-park

Qian, P., Schoenau, J. J., & Ziadi, N. (2008). Ion Supply Rates Using Ion-Exchange Resins. In *Soil Sampling and Methods of Analysis*.

Quintana-Seguí, P., Martin, E., Sánchez, E., Zribi, M., Vennetier, M., Vicente-Serrano, S., & Vidal, J.-P. (2016). Sub-chapter 1.3.3. Drought: observed trends, future projections. In *The Mediterranean region under climate change* (pp. 123–131). https://doi.org/10.4000/books.irdeditions.23157

Ramos, A., Pereira, M. J., Soares, A., Rosário, L. Do, Matos, P., Nunes, A., ... Pinho, P. (2015). Seasonal patterns of Mediterranean evergreen woodlands (Montado) are explained by long-term precipitation. *Agricultural and Forest Meteorology*, *202*, 44–50. https://doi.org/10.1016/j.agrformet.2014.11.021

Rastogi, M., Singh, S., & Pathak, H. *Emission of carbon dioxide from soil.* , 82 § (2002).

Reichstein, M., Tenhunen, J. D., Roupsard, O., Ourcival, J.-M., Rambal, S., Dore, S., & Valentini, R. (2002). Ecosystem respiration in two Mediterranean evergreen Holm Oak forests: drought effects and decomposition dynamics. *Functional Ecology*, *16*(1), 27–39. https://doi.org/10.1046/j.0269-8463.2001.00597.x

Ren, C., Chen, J., Lu, X., Doughty, R., Zhao, F., Zhong, Z., ... Ren, G. (2018). Responses of soil total microbial biomass and community compositions to rainfall reductions. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2017.09.028

Ren, C., Zhao, F., Shi, Z., Chen, J., Han, X., Yang, G., ... Ren, G. (2017). Differential responses of soil microbial biomass and carbon-degrading enzyme activities to altered precipitation. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2017.08.002

Reyes-García, V., Fernández-Llamazares, Á., Guèze, M., Garcés, A., Mallo, M., Vila-Gómez, M., &

Vilaseca, M. (2016). Local indicators of climate change: The potential contribution of local knowledge to climate research. *Wiley Interdisciplinary Reviews. Climate Change*, *7*(1), 109–124. https://doi.org/10.1002/wcc.374

Rodríguez, A., Curiel Yuste, J., Rey, A., Durán, J., García-Camacho, R., Gallardo, A., & Valladares, F. (2017). Holm oak decline triggers changes in plant succession and microbial communities, with implications for ecosystem C and N cycling. *Plant and Soil*, *414*(1–2), 247–263. https://doi.org/10.1007/s11104-016-3118-4

Rodríguez, A., Durán, J., Rey, A., Boudouris, I., Valladares, F., Gallardo, A., & Yuste, J. C. (2019). Interactive effects of forest die-off and drying-rewetting cycles on C and N mineralization. *Geoderma*, *333*(March 2018), 81–89. https://doi.org/10.1016/j.geoderma.2018.07.003

Salazar, A., Sulman, B. N., & Dukes, J. S. (2018). Microbial dormancy promotes microbial biomass and respiration across pulses of drying-wetting stress. *Soil Biology and Biochemistry*, *116*(October 2017), 237–244. https://doi.org/10.1016/j.soilbio.2017.10.017

Samaniego, L., Thober, S., Kumar, R., Wanders, N., Rakovec, O., Pan, M., ... Marx, A. (2018). Anthropogenic warming exacerbates European soil moisture droughts. *Nature Climate Change*, *8*(5), 421–426. https://doi.org/10.1038/s41558-018-0138-5

Sardans, J., & Peñuelas, J. (2005a). Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. *Soil Biology and Biochemistry*, *37*(3), 455–461. https://doi.org/10.1016/j.soilbio.2004.08.004

Sardans, J., & Peñuelas, J. (2005b). Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. *Soil Biology and Biochemistry*, *37*(3), 455–461. https://doi.org/10.1016/J.SOILBIO.2004.08.004

SARDANS, J., & PEÑUELAS, J. (2007). Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. *Functional Ecology*, *21*(2), 191–201. https://doi.org/10.1111/j.1365-2435.2007.01247.x

Sardans, J., Peñuelas, J., & Estiarte, M. (2008). Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. *Applied Soil Ecology*, *39*(2), 223–235. https://doi.org/10.1016/J.APSOIL.2007.12.011

Sardans, J., Peñuelas, J., & Ogaya, R. (2008). Experimental drought reduced acid and alkaline phosphatase activity and increased organic extractable P in soil in a Quercus ilex Mediterranean forest. *European Journal of Soil Biology*. https://doi.org/10.1016/j.ejsobi.2008.09.011

Sardans, Jordi, & Pen, J. (2008). *Drought-Induced Changes in C and N Stoichiometry in a Quercus ilex Mediterranean Forest*. *54*(5), 513–522.

Sardans, Jordi, & Peñuelas, J. (2004). Increasing drought decreases phosphorus availability in an evergreen Mediterranean forest. *Plant and Soil*, *267*(1–2), 367–377. https://doi.org/10.1007/s11104-005-0172-8

Sardans, Jordi, Peñuelas, J., & Estiarte, M. (2007). Seasonal patterns of root-surface phosphatase activities in a Mediterranean shrubland. Responses to experimental warming and drought. *Biology and Fertility of Soils*, *43*(6), 779–786. https://doi.org/10.1007/s00374-007-0166-1

Sardans, Jordi, Rodà, F., & Peñuelas, J. (2004). Phosphorus limitation and competitive capacities of Pinus halepensis and Quercus ilex subsp. rotundifolia on different soils. *Plant Ecology Formerly `Vegetatio'*, *174*(2), 307–319. https://doi.org/10.1023/b:vege.0000049110.88127.a0

Schaufler, G., Kitzler, B., Schindlbacher, A., Skiba, U., Sutton, M. A., & Zechmeister-Boltenstern, S. (2010). Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature. *European Journal of Soil Science*, *61*(5), 683–696. https://doi.org/10.1111/j.1365-2389.2010.01277.x

Schwilch et al, 2016. (2016). *Soil Threats in Europe: Status, methods, drivers, and effects on ecosystem services*.

Shvaleva, A, Cruz, C., Castaldi, S., Rosa, A. P., Chaves, M. M., & Pereira, J. S. (2011). *Soil-atmosphere greenhouse gases (CO2, CH4 and N2O) exchange in evergreen oak woodland in southern Portugal*. *2011*(10), 471–477.

Shvaleva, Alla, Costa e Silva, F., Costa, J. M., Correia, A., Anderson, M., Lobo-do-Vale, R., ... Cruz, C. (2014). Comparison of methane, nitrous oxide fluxes and CO2 respiration rates from a

Mediterranean cork oak ecosystem and improved pasture. *Plant and Soil*, *374*(1–2), 883–898. https://doi.org/10.1007/s11104-013-1923-6

Singh, B. (2011). The nitrogen cycle: implications for management, soil health, and climate change. In *Soil Health and Climate Change* (p. Chapter 6). https://doi.org/10.2136/sssaj2012.0004br

Smith, K. A., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., & Rey, A. (2018). Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science*, *69*(1), 10–20. https://doi.org/10.1111/ejss.12539

SRIVASTAVA, P., SINGH, R., TRIPATHI, S., SINGH, P., SINGH, S., SINGH, H., ... MISHRA, P. K. (2017). Soil Carbon Dynamics Under Changing Climate—A Research Transition from Absolute to Relative Roles of Inorganic Nitrogen Pools and Associated Microbial Processes: A Review. *Pedosphere*, *27*(5), 792–806. https://doi.org/10.1016/S1002-0160(17)60488-0

Stark, J. M., & Firestone, M. K. (1995). Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology*, *61*(1), 218–221. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16534906%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1388328

Stuart-Haëntjens, E., De Boeck, H. J., Lemoine, N. P., Mänd, P., Kröel-Dulay, G., Schmidt, I. K., ... Smith, M. D. (2018). Mean annual precipitation predicts primary production resistance and resilience to extreme drought. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2018.04.290

Teuling, A. J. (2018). A hot future for European droughts. *Nature Climate Change*, *8*(5), 364–365. https://doi.org/10.1038/s41558-018-0154-5

UN Environmental Programme. (2017). Population and Development | UNEPMAP QSR. Retrieved August 9, 2019, from Mediterranean 2017 Quality Status Report website: https://www.medqsr.org/population-and-development

Unger, S., Máguas, C., Pereira, J. S., David, T. S., & Werner, C. (2010). The influence of precipitation pulses on soil respiration - Assessing the " Birch effect" by stable carbon isotopes. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2010.06.019

Unger, S., Máguas, C., Pereira, J. S., David, T. S., & Werner, C. (2012). Interpreting post-drought rewetting effects on soil and ecosystem carbon dynamics in a Mediterranean oak savannah. *Agricultural and Forest Meteorology*, *154–155*, 9–18. https://doi.org/10.1016/j.agrformet.2011.10.007

Ussiri, D., & Lal, R. (2013). *Soil Emission of Nitrous Oxide and its Mitigation*. https://doi.org/10.1007/978-94-007-5364-8

Valentini, R., Kovats, R. S., Bouwer, L. M., Georgopoulou, E., Jacob, D., Martin, E., ... Soussana, J. (2014). 2014: Europe. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, 1267–1326. https://doi.org/10.1017/CBO9781107415386.003

Van Groenigen, J. W., Huygens, D., Boeckx, P., Kuyper, T. W., Lubbers, I. M., Rütting, T., & Groffman, P. M. (2015). The soil n cycle: New insights and key challenges. *Soil*, *1*(1), 235–256. https://doi.org/10.5194/soil-1-235-2015

Veraart, A. J., Steenbergh, A. K., Ho, A., Kim, S. Y., & Bodelier, P. L. E. (2015). Beyond nitrogen: The importance of phosphorus for CH4 oxidation in soils and sediments. *Geoderma*, *259–260*, 337–346. https://doi.org/10.1016/j.geoderma.2015.03.025

Vicente-Serrano, S. M., Lopez-Moreno, J. I., Beguería, S., Lorenzo-Lacruz, J., Sanchez-Lorenzo, A., García-Ruiz, J. M., ... Espejo, F. (2014). Evidence of increasing drought severity caused by temperature rise in southern Europe. *Environmental Research Letters*, *9*(4). https://doi.org/10.1088/1748-9326/9/4/044001

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(14), 5266–5270. https://doi.org/10.1073/pnas.1320054111

Wahren, H. (n.d.). itex_site1-17.jpg (3008×2000). Retrieved August 18, 2019, from https://jscamacresearch.files.wordpress.com/2011/04/itex_site1-17.jpg

Walkiewicz, A., & Brzezińska, M. (2019). Interactive effects of nitrate and oxygen on methane oxidation in three different soils. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2019.03.001

Wrage-Mönnig, N., Horn, M. A., Well, R., Müller, C., Velthof, G., & Oenema, O. (2018). The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biology and Biochemistry*, *123*(April), A3–A16. https://doi.org/10.1016/j.soilbio.2018.03.020

Wrage, N., Velthof, G. L., van Beusichem, M. ., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry*, *33*, 1723–1732.

WWF. (2004). WWF cork oak landscapes programme. In *WWF Mediterranean Programme*. Retrieved from http://assets.panda.org/downloads/factsheetcork.pdf

Xiang, S.-R., Doyle, A., Holden, P. A., & Schimel, J. P. (2008). Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. *Soil Biology and Biochemistry*, *40*(9), 2281–2289. https://doi.org/10.1016/j.soilbio.2008.05.004

Xu, W., & Yuan, W. (2017). Responses of microbial biomass carbon and nitrogen to experimental warming: A meta-analysis. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2017.08.033

Yang, Y., Halbritter, A. H., Klanderud, K., Telford, R. J., Wang, G., & Vandvik, V. (2018). Transplants, Open Top Chambers (OTCs) and Gradient Studies Ask Different Questions in Climate Change Effects Studies. *Frontiers in Plant Science*. https://doi.org/10.3389/fpls.2018.01574

Yu, Z., Wang, G., & Marschner, P. (2014). Drying and rewetting - Effect of frequency of cycles and length ofmoist period on soil respiration and microbial biomass. *European Journal of Soil Biology*, *62*, 132–137. https://doi.org/10.1016/j.ejsobi.2014.03.007

Zaehle, S. (2013). Terrestrial nitrogen–carbon cycle interactions at the global scale. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130125. https://doi.org/10.1098/RSTB.2013.0125

7 ANNEX

I. Table summarizing compounds used in lab analyses, the components that were used to make them, and the available lot/batch information. “*” indicates information not available.

Compound/Chemical	Composition	Company	Lot/Batch
Potassium Chloride	-	Honewell Fluka	*
Citrate	Trisodium citrate	VWR Chemicals Intl	16B290011
	NaOH	VWR Chemicals Intl	16C100018
Salicylate	Sodium salicylate	Merck KGaA	K49445801
	Sodium pentanitroferricyanacide (III)-dihydrate	Alfa Aesar	Y23B027
Hypochlorite	Sodium phosphate	ITW Reagents	0000652924
	NaOH	VWR Chemicals Intl	16C100018
	Sodium hypochlorite	ITW Reagents	5Z016250
Sulfuric Acid	-	Sigma-Aldrich	SZBB1870V
Potassium Nitrate	-	*	*
Ammonium chloride	-	*	*
Devarda’s Alloy	-	Merek KGaA	K47839041
Ammonium Molybdate	-	*	*
Tin (II) Chloride dehydrate	-	*	*

II. GLS model results for ammonium (NH_4)

Model Formula

gls(NH4.N~OTC*RE*Season, weights=varComb(varPower(form=~fitted(.)|Treatment*Season)), correlation=corCompSymm(form=~1|Tree), method="REML",na.action=na.omit, data=IEMGamir

GLS Model

Variable		**Estimates**	**Std. Error**	***P***
Treatment* + *Season	*(Intercept)*	0.1060	0.0076	0.0000
	OTC	0.0505	0.0173	0.0039
	RE	0.0611	0.0174	0.0005
	OTC:RE	-0.0411	0.0278	0.1391
	Season			
	Spring	-0.0317	0.0137	0.0213
	Summer	-0.0578	0.0080	0.0000
Interactions	*RE:Summer*	-0.0749	0.0173	0.0000
	OTC:Spring	-0.0563	0.0229	0.0145
	OTC:Summer	-0.0473	0.0184	0.0105

III. GLS model results for inorganic N (NH_4NO_3)

Model Formula

gls(NH4NO3.N~OTC*RE*Season, weights=varComb(varPower(form=~fitted(.)|Treatment*Season)), correlation=corCompSymm(form=~1|Tree), method="REML",na.action=na.omit, data=IEMGamir)

GLS Model

Variable		**Estimates**	**Std. Error**	P_{GLS}
Treatment* + *Season	*(Intercept)*	0.4118	0.045	0.000
	OTC	0.0575	0.067	0.391
	RE	-0.0296	0.054	0.582
	OTC:RE	-0.0287	0.084	0.732
	Season			
	Spring	-0.246	0.048	0.000
	Summer	-0.311	0.045	0.000
Interactions	*RE:Spring*	0.1683	0.072	0.023

IV. GLS model results for nitrate (NO_3)

Model Formula				
gls(NO3~OTC*RE*Season, weights=varComb(varPower(form=~fitted(.)|Treatment*Season)), correlation=corCompSymm(form=~1|Tree), method="REML",na.action=na.omit, data=IEMGamir)				
GLS Model				
Variable		**Estimates**	**Std. Error**	P_{GLS}
Treatment* + *Season	*(Intercept)*	0.0306	0.0436	0.000
	OTC	-0.0077	0.0575	0.894
	RE	-0.0906	0.0495	0.066
	OTC:RE	0.0274	0.0721	0.704
	Season			
	Spring	-0.2164	0.0437	0.000
	Summer	-0.2530	0.0432	0.000
Interactions	*RE:Season*			

V. GLS model results for inorganic phosphate (PO_4)

Model Formula				
gls(PO4.P~OTC*RE*Season, weights=varComb(varPower(form=~fitted(.)|Treatment*Season)), correlation=corCompSymm(form=~1|Tree), method="REML",na.action=na.omit, data=IEMGamir)				
GLS Model				
Variable		**Estimates**	**Std. Error**	P_{GLS}
Treatment* + *Season	*(Intercept)*	0.0019	0.0003	0.0000
	OTC	0.0001	0.0004	0.8034
	RE	0.0038	0.0008	0.0000
	OTC:RE	-0.0010	0.0010	0.3258
	Season			
	Spring	0.0030	0.0007	0.0000
	Summer	-0.0012	0.0003	0.0003
Interactions	*RE:Spring*	-0.0038	0.0011	0.0006
	RE:Summer	-0.0023	0.0008	0.0084
	RE:Season			

VI. LMM results for carbon dioxide (CO_2)

<table>
<tr><td colspan="6">Model Formula
lmer(CO2~OTC*RE*Season+(1|Tree), na.action=na.omit, data=Gases)</td></tr>
<tr><td rowspan="9">Fixed Effects</td><td>Variable</td><td>Estimates</td><td>Std. Error</td><td>D.F</td><td><i>Pr(>|t|)</i></td></tr>
<tr><td><i>(Intercept)</i></td><td>969.32</td><td>303.65</td><td>262.97</td><td>0.0015**</td></tr>
<tr><td><i>OTC</i></td><td>115.21</td><td>416.29</td><td>231.50</td><td>0.782210</td></tr>
<tr><td><i>RE</i></td><td>306.47</td><td>417.33</td><td>262.96</td><td>0.463377</td></tr>
<tr><td><i>OTC:RE</i></td><td>18.65</td><td>579.95</td><td>229.86</td><td>0.974369</td></tr>
<tr><td colspan="5">Seasons</td></tr>
<tr><td><i>Spring</i></td><td>1597.80</td><td>422.04</td><td>233.31</td><td>0.0002***</td></tr>
<tr><td><i>Summer</i></td><td>253.34</td><td>416.29</td><td>231.50</td><td>0.5433</td></tr>
<tr><td><i>Winter</i></td><td>1380.44</td><td>422.04</td><td>233.31</td><td>0.0012**</td></tr>
<tr><td rowspan="3">Random Effects</td><td>Variable</td><td>Variance</td><td colspan="3">Standard Dev.</td></tr>
<tr><td><i>Tree</i></td><td>7804</td><td colspan="3">88.34</td></tr>
<tr><td><i>Residual</i></td><td>1467461</td><td colspan="3">1211.39</td></tr>
</table>

VII. LMM results for nitrous oxide (N_2O)

<table>
<tr><td colspan="6">Model Formula
lmer(sqrt(N2O)~OTC*RE*Season+(1|Tree), na.action=na.omit, data=Gases)</td></tr>
<tr><td rowspan="9">Fixed Effects</td><td>Variable</td><td>Estimates</td><td>Std. Error</td><td>D.F</td><td><i>Pr(>|t|)</i></td></tr>
<tr><td><i>(Intercept)</i></td><td>11.78</td><td>1.71</td><td>138.00</td><td>2.02e-10 ***</td></tr>
<tr><td><i>OTC</i></td><td>-0.26</td><td>2.19</td><td>138.00</td><td>0.907</td></tr>
<tr><td><i>RE</i></td><td>-0.0076</td><td>2.24</td><td>138.00</td><td>0.9973</td></tr>
<tr><td><i>OTC:RE</i></td><td>-0.074</td><td>2.96</td><td>138.00</td><td>0.9802</td></tr>
<tr><td colspan="5">Seasons</td></tr>
<tr><td><i>Spring</i></td><td>5.431752</td><td>2.3473</td><td>138.00</td><td>0.0221 *</td></tr>
<tr><td><i>Summer</i></td><td>-2.750228</td><td>2.424362</td><td>138.00</td><td>0.2586</td></tr>
<tr><td><i>Winter</i></td><td>-2.616184</td><td>2.28571</td><td>138.00</td><td>0.2544</td></tr>
<tr><td rowspan="3">Random Effects</td><td>Variable</td><td>Variance</td><td colspan="3">Standard Dev.</td></tr>
<tr><td><i>Tree</i></td><td>0.00</td><td colspan="3">0.00</td></tr>
<tr><td><i>Residual</i></td><td>20.57</td><td colspan="3">4.536</td></tr>
</table>

VIII. LMM results for methane (CH_4)

Model Formula **lmer(CH4~OTC*RE*Season+(1|Tree), na.action=na.omit, data=Gases)**					
Mixed Model					
Fixed Effects	**Variable**	**Estimates**	**Std. Error**	**D.F**	***Pr(>|t|)***
	(Intercept)	-0.199	0.047	256.3	3.28e-05 ***
	OTC	0.087	0.065	231.8	0.1812
	RE	0.088	0.066	256.3	0.1871
	OTC:RE	-0.153	0.091	231.2	0.0960 .
	Seasons				
	Spring	-0.055	0.065	231.8	0.4020
	Summer	0.097	0.064	230.7	0.1331
	Winter	0.0523	0.064	230.7	0.4155
Random Effects	**Variable**	**Variance**	**Standard Dev.**		
	Tree	0.002674	0.05171		
	Residual	0.037025	0.19242		