Supporting Information

Plant-available N:P alters root litter N recycling in a Mediterranean tree–grass ecosystem

Richard K. F. Nair*, **Kendalynn A. Morris**, **Mirco Migliavacca**, **Gerado Moreno**, and **Marion Schrumpf**

10.1002/jpln.202000097

jpln202000097-sup-0001-misc_information.pdf

SUPPLEMENTARY METHODS

For leaching measurements, we installed resin ion exchange bags beneath the ingrowth cores (including the isotope-labelled cores, although these were not measured) at installation. We used 6.86 mg of Dowex Marathon MR-3 mixed-bed resin in each bag, which was constructed of a square of nylon fabric placed around a zip tie (diameter approximately 4 cm) and sealed by drawing tight around a second zip tie. Bags were installed within the base of 2/3 of the mesocosm in each micro-site and recovered in May 2017. Resins were kept moist before installation and after collection and were extracted for exchangeable N by cleaning and shaking in 2M KCl following standard methodology; following three extractions in 20ml 2M KCl and filtering through Whatman No.1 filter paper, the resulting filtrate was analyzed for total N content.

However, due to a combination of an unexpectedly fast dry-down at the site, and laboratory accident, we were only able to recover a minority (~12%) of individual resin bags for analysis. We therefore pooled these for the whole experiment rather than over-interpret the sparse data. The coarse estimates presented of total leaching assume that due to pressure from the internal zip tie, resin bags occupied the entire 4 cm area beneath the core and a value could be obtained on average by pooling surviving samples from all treatments.

DIFFERENCES BETWEEN ISOTOPE LABELLED AND CONTROL ROOTS

From our measurements, root N contents were slightly higher in the isotope labelled treatment ($P < 0.05$, Figure S1) than the control treatment. This may have been due to variation in N availability from source litter which had slightly higher N content for some of the labelled cores.

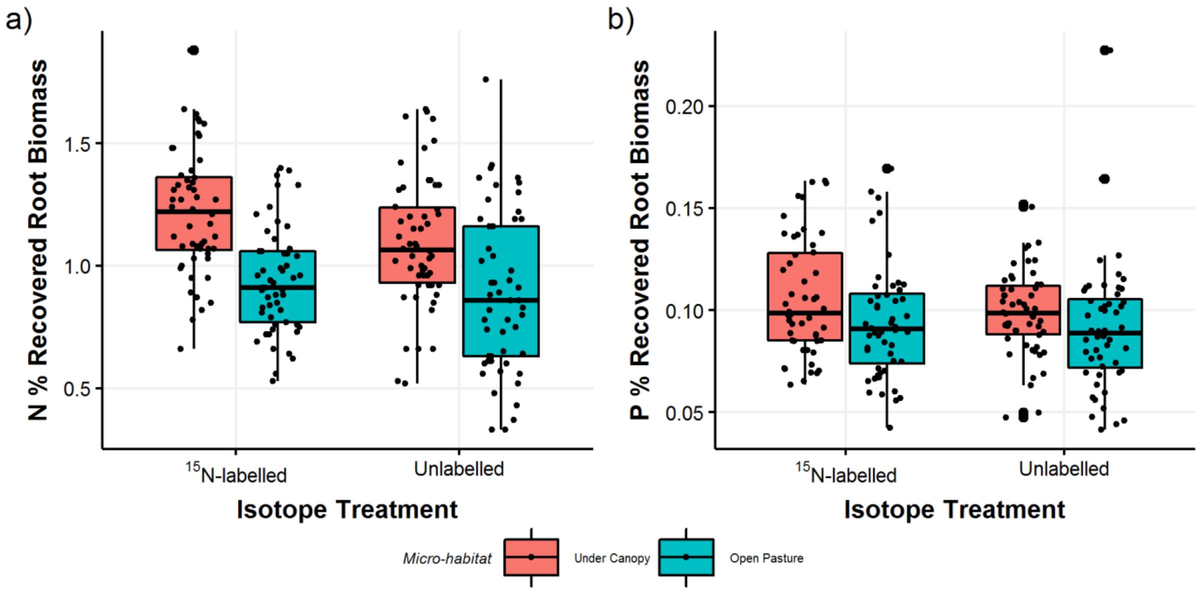

Figure S1. N and P contents depending on isotope treatment.

DIFFERENCES BETWEEN MICROSITES AND REPRESENTATIVENESS OF UNDERLYING PATTERNS

Micro-sites were highly variable and there were not strong trends in total root harvest within individual microsites nor beneath individual trees (Figure S2), supporting expectations of high species turnover and variability. This supported our assumption that the sampling design, by necessity pseudo-replicated, nonetheless covered real variation within the site and could be used to assess the effects of nutrient treatments.

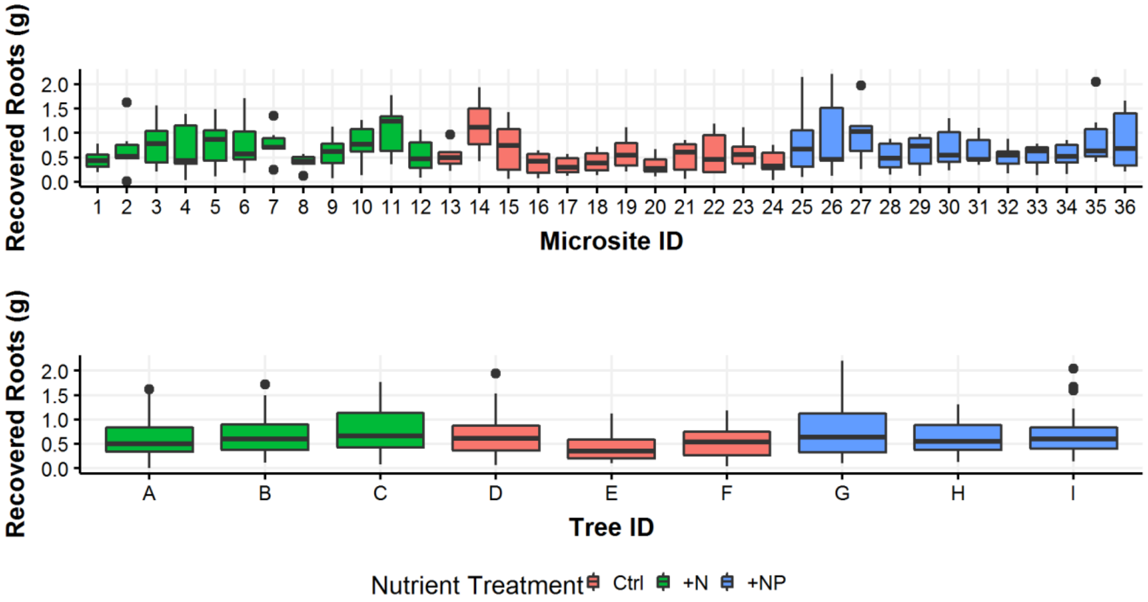

Figure S2. Micro-site Heterogeneity. There were no significant differences between individual sites nor trees.

ROOT NITROGEN AND PHOSOPHORUS CONTENTS

Raw data on N and P concentrations (Figure S3) was used to calculate N:P ratios in Figure 2 in the main manuscript. These data were more consistent between treatments than the ratio between them. Generally, NP treatment effects were more visible in May and N treatment effects more visible in December. This may relate to the different availability of the ionic forms discussed in the main manuscript.

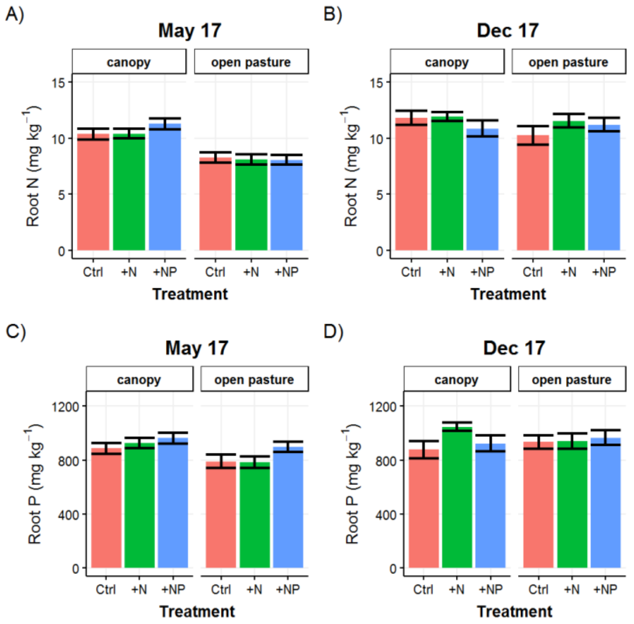

Figure S3. Nitrogen and Phosphorus contents of roots in May and December. The effects of N additions were more evident in December and the effects of NP additions more evident in May.

SOIL NITROGEN AND PHOSPHORUS CONTENTS

We show here soil extractable N and P. N content for these measurements were 2M KCl extracts, and P content Olsen-P. These data are not directly comparable to K_2SO_4-extractable N in the main manuscript due to the different extractant used, and as these soil ratios were from inorganic N only, while our extractable N in this paper is both inorganic and organic N.

We used bulked soil from a 13 cm deep ingrowth core while the soil N:P came from 5 cm ’undisturbed’ cores with a smaller sample size and total N concentrations around twice which we calculated from the ingrowth cores. In similar Californian systems, most ecosystem N is found in the top 4 cm of soil (Jackson et al. 1989)

Micro-habitat effects were more evident in ingrowth core data in this paper, likely because K_2SO_4 is a better matrix for extracting organic components and the dominant difference between micro-habitats is the soil organic matter content.

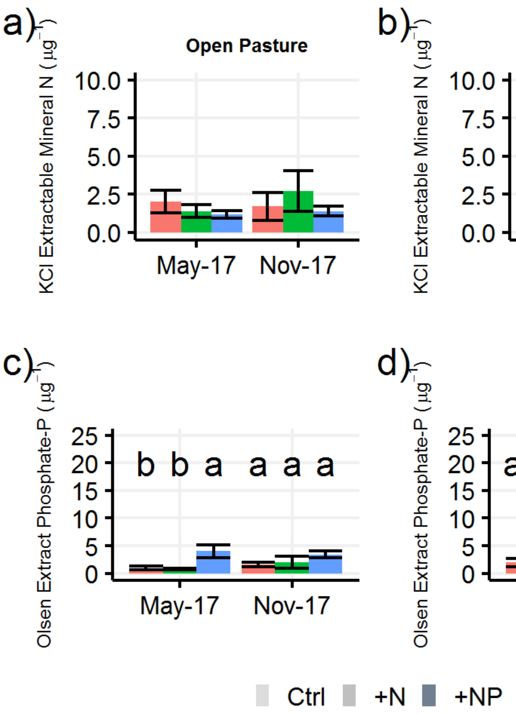

Figure S4: Extractable Nitrogen and Phosphorus contents of 5cm topsoil in May and December. Letters show Tukey HSD from ANOVA within each date. Errors are standard error.

VEGETATION COVER AND SPECIES RICHNESS CHANGE

From vegetation surveys (not paired to our sampling sites), we could validate some of the explanations for our results. Legume percentage cover had decreased in the N treatment (Figure S5 and overall species richness had declined in both fertilized treatments (Figure S6). This agrees with general understanding of fertilization effects and a suppression of species richness by nutrient availability.

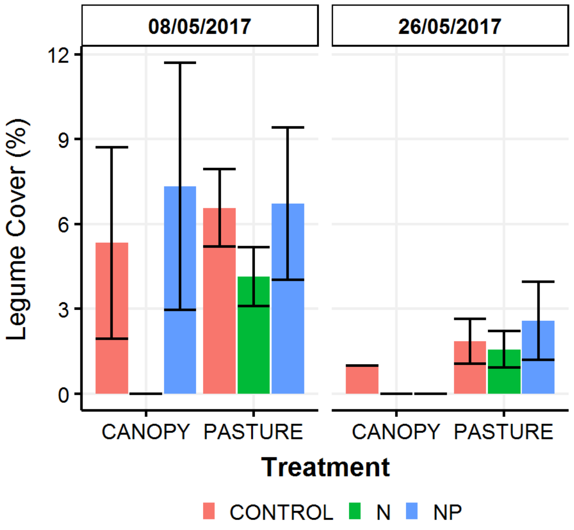

Figure S5. Legume Percentage Cover in May 2017 between two samplings (a) 8th May and (b) 25 May. Per treatment, these are n = 3 (canopy) and n = 7 (open pasture) and are not paired directly to the sites of root measurements. Legume abundance is suppressed in the N treatment, particularly heavily under canopies. Errors are standard deviation

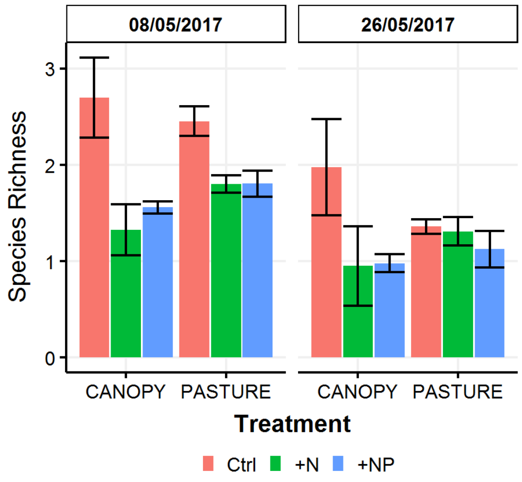

Figure S6. Species Richness (Menhinick's Index) of the herbaceous layer in May 2017 between two samplings (a) 8th May and (b) 25 May. Per treatment, these are n = 3 (canopy) and n=7 (open pasture) and are not paired directly to the sites of root measurements. Species richness is suppressed by nutrient treatments.

N:P RATIOS OF LEAVES

Leaf N:P ratios are commonly used as a reliable index of N and P limitation (Ostertag and DiManno 2016) and we also conducted vegetation surveys above-ground at our site. These were not performed in the same sampling scheme as the root measurements reported in the rest of this paper, instead being sampled once or twice for each year since before the fertilization until 2018, at the 'peak' of the growing season before the summer dry down. In this data there was no separation between canopy and open pasture locations. An unbiased sample of green leaves was ground and measured using the same methods as the root samples in the main manuscript.

As these leaf nutrient contents were not easily transformed to normal distribution, they were analysed with non-parametric Wilcoxen 2-way signed rank test at each sampling date. NP ratio was however transformable to a normal distribution, and for this we fit a mixed effects model, treating date of sampling as a random effect as these did not resample the same areas and were a relatively sparse time series not particularly suitable for a autocorrelated error structure.

Before fertilization (in 2014), leaf N was similar between treatments and P slightly lower in NP (the opposite of later treatment effects) and resulting in a higher N:P ratio. N and NP additions (represented by a red line in figure S7) increased N contents and N and P contents respectively. N contents were less consistently elevated in 2017 unlike P contents which were significantly higher in the NP treatments except in the first sampling in

2015. N:P ratio was elevated the N treatments in 2015 but later in the experiment this difference had declined; in the mixed effect model there was a significant effect ($P < 0.001$) of the N treatment on this ratio. In the NP treatment, N:P ratio was in most sampling dates statistically similar to the control treatment, but by early May 2016 and May 2017 lower in the NP treatment, indicating a higher relative P availability compared to N.

While this above-ground data is available on a different spatial scale and distribution than the below-ground data otherwise presented in this paper it suggests a similar overall pattern; the initial treatment effects, particularly of the N treatment, gradually declining through time. During the period of our root stoichiometry data (2017) the enhanced P availability and less distinct N availability in treatments seen elsewhere is also visible above-ground. Unfortunately similar data were not collected in the December 'fallow' period, where we could detect effects of the N treatment. This highlights the need for out-of-season measurements to understand phenological functioning of such multiple resource limited systems.

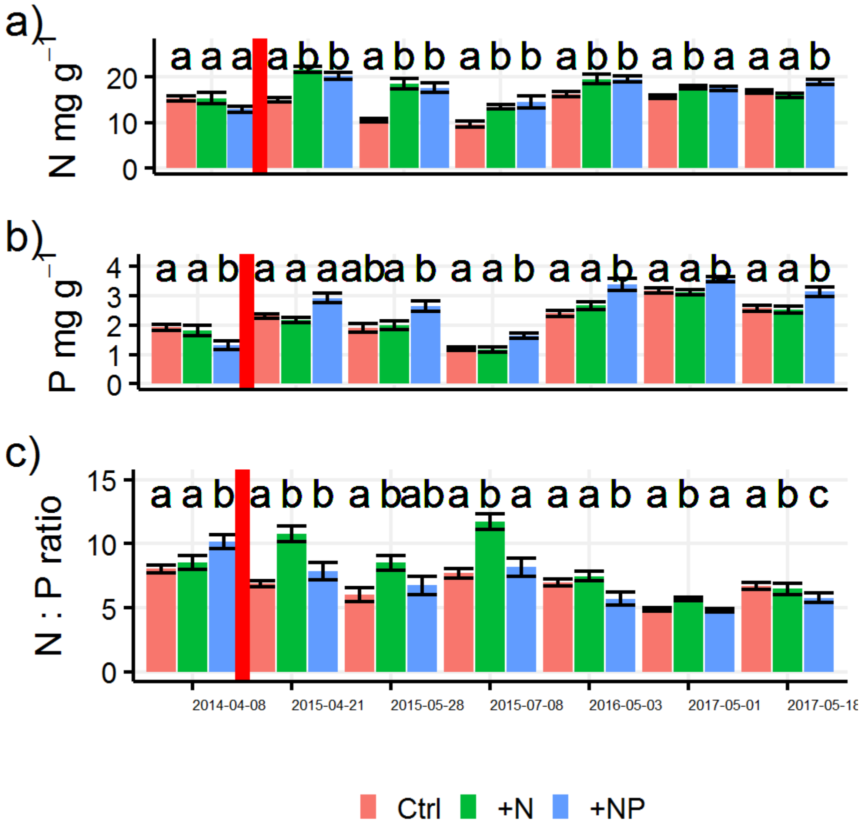

Figure S7. N and P content of leaves and NP ratio, letters indicate Wilcoxen 2-way signed rank test within sampling date. Letters are Tukey HSD for ANOVA conducted within-date.