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Re-allocation of nitrogen and phosphorus from roots drives regrowth of grasses and sedges after defoliation under deficit irrigation and nitrogen enrichment

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Summary

1. Re-allocation of nutrients from roots to shoots is essential for plant regrowth in grasslands, particularly in nutrient-poor conditions. However, the response of root nutrient re-allocation to changes in nitrogen (N) and water availability remains largely unknown.

2. Using a novel ^{15}N and ^{32}P labelling technique, we quantified the contribution of N and phosphorus (P) to shoot regrowth from either root re-allocation or direct soil uptake for perennial grasses exposed to high-frequency deficit irrigation (HFDI) and N addition.

3. Without N addition, HFDI showed no impact on uptake and re-allocation of N and P, likely due to unaffected soil N availability and a greater diffusion barrier offsetting increased accumulation in plant-available soil P. With N addition, HFDI increased plant N rather than P uptake, because of increasing soil N availability instead of P under combined HFDI and N addition. The HFDI decreased both N and P re-allocation with N addition, possibly due to exhaustion of nutrient reserves in roots that were re-allocated aboveground. Re-allocation contributed 48-97% of N and 58-79% of P required during the first two weeks of shoot regrowth.

4. *Synthesis*. Our results highlight the importance of N and P re-allocation from roots to buffer against changes in soil N and P availability and to maintain N:P ratio in shoot regrowth.

Introduction

Nitrogen (N) and phosphorus (P) are co-limiting nutrients of net primary productivity in most terrestrial ecosystems (Harpole et al., 2011; Fay et al., 2015). To satisfy N and P demand for shoots, plants take up nutrients from the soil but also recycle nutrients internally through resorption and re-allocation from storage organs (*e.g.*, roots), which allows the reuse of nutrients for new growth (Freschet and Roumet, 2017; Klimešová et al., 2018). Re-allocation of N and P from roots is an important nutrient-use strategy for plant growth when nutrient availability to plants is low or when aboveground parts are removed or damaged (*e.g.*, by grazing and fire) (Millard & Grelet, 2010; Pereira-Silva et al., 2019). In grasslands in particular, plants are often adapted to recover from herbivory through shoot regrowth whereby nutrient resorption and re-allocation processes play an important role (Zhang et al., 2020, 2021). Therefore, efficient N and P uptake from soils and re-allocation from plant roots both strongly affect plant growth, survival and reproduction, particularly under stressful conditions such as water or nutrient limitation (Reich, 2014; Schönbeck et al., 2020). Yet, how much plants rely on N and P uptake from soil *vs.* re-allocation from roots for their regrowth remains largely unknown. Better understanding of N and P recycling under changing environmental conditions (*e.g.*, intermittent rainfall) is particularly critical for worldwide plant production, given that large parts of terrestrial biomes may become increasingly limited by N- and/or P under hotter and drier conditions (Ault, 2020; Du et al., 2020).

Reduced soil moisture decreases soil nutrient availability directly by decreasing nutrient diffusion rates and indirectly by decreasing nutrient release from decomposition of soil organic matter due to constrained microbial activity (Lambers et al., 2008; Ullah et al., 2019). For instance, reduced soil moisture decreased plant N and P uptake due to limited nutrient transport activity both in soils and plants (Khasanova et al., 2013; Dijkstra et al., 2015). In order to maintain productivity under low soil moisture, plants may need to increasingly rely on internal recycling of N and P from storage organs. Additionally, reduced soil moisture was found to decrease plant-available soil P concentration to a larger degree than mineral N in soils (Dijkstra et al., 2012; He & Dijkstra, 2014); hence, reliance on P recycling may be higher than for N. However, the role of N and P re-allocation from roots to support plant regrowth under low soil moisture remains largely unknown.

Nitrogen enrichment, as widely experienced in terrestrial ecosystems, can alleviate plant N limitation but potentially intensify P limitation (Li et al., 2016); this may have important stoichiometric implications for plants. With increased N supply, plants rely more on N uptake from the soil compared with N recycling (Shi et al., 2016), and re-allocation of N from roots may decrease with N addition. However, N addition would increase plant demand for P, shifting nutrient limitation from N towards P (Li et al., 2016; Deng et al., 2017). If this increased plant demand for P cannot be met with P uptake from the soil (*e.g.*, under low soil moisture conditions), then plants subjected to greater N availability may have to rely more on re-allocation of P from roots to shoots to balance nutrients for new shoot growth. At the same time, root to shoot biomass ratio may increase as plants invest relatively more in root biomass in such N-rich but water-deficit circumstances (Wang et al., 2021a). The capacity to balance N and P in new shoot growth by controlling N and P uptake from the soil *vs.* re-allocation from roots may therefore be of particularly importance under low soil moisture conditions, but as far as we know, this has never been assessed.

Stoichiometric N:P stability is an essential trait for maintaining plant growth under unbalanced N and P supply or under nutrient-deficit conditions. For instance, decreases in N and P availability as induced by low soil moisture favoured plant species with stoichiometric N:P stability which generally exhibited a greater nutrient-foraging capacity and thus stronger plant dominance (Yu et al., 2010, 2015). In such circumstances, plants may also adjust re-allocation of N and P from roots to shoots to maintain N:P ratio. Alternatively, plant species with flexible N:P ratios were found to be more resistant to low soil moisture possibly linked to mycorrhizal symbiosis (Mariotte et al., 2017). The stoichiometric N:P ratio in plants can also indicate relative N and P limitations as induced by variations in N and P supply under environmental perturbations (Güsewell, 2005; Yan et al., 2017). With increased soil N supply, intensified plant P limitation as evidenced by higher N:P ratios therefore suggests a greater need for the re-allocation of P than that of N from roots.

Here, we used a novel isotopic dual-labelling technique (^{15}N and ^{32}P) to quantify plant N and P uptake from the soil and internal re-allocation from roots to shoot regrowth in an intact plant-soil system as sampled from a perennial grassland. The effects of low soil moisture (*i.e.* deficit irrigation) and N addition were further assessed on uptake and re-allocation of N and P and their contribution to plant regrowth. We hypothesized that 1) deficit irrigation increases plant reliance on re-allocation of P, more so than N, from roots to shoots, to compensate for the decreases in plant P and N uptake from the soil; 2) N addition decreases plant reliance on re-allocation of N due to exogenous soil N supply but increases reliance on re-allocation of P under deficit irrigation because of limited soil P availability and uptake; and 3) deficit irrigation and N addition synergistically interact to increase N:P ratios from soil uptake and thus reduce N:P ratios from root re-allocation.

2. Materials and methods

2.1. Experimental design

In October 2019, 20 intact plant-soil cores were collected from a grassland at Westwood Farm (33°59′46″S, 150°39′16″E), NSW, Australia. The plant community of this grassland is dominated by the C4 grass *Paspalum dilatatum* Poir., contributing to 71% of shoot biomass, but it also includes the C4 sedge *Cyperus brevifolius* and grass *Setaria incrassata*, and the C3 grass *Microlaena stipoides*. These species were in vegetative stage at the time of field sampling. Mean annual precipitation at the site is 790 mm and long-term average monthly temperatures are 10.4 °C and 23.0 °C in July and January, respectively. The pasture site is moderately grazed by cattle, and there is a history of no fertiliser being added to the soil.

After field sampling, the 20 intact cores were excavated to the same size (150 mm in diameter, 100 mm in height) and placed in plastic pots (150 mm diameter, 130 mm height). This grassland community is shallow-rooted due to an acidic subsoil layer, and root density sharply declines with depth with 85% of the root biomass allocated in the upper 50 mm of the top 150 mm soil (Canarini et al., 2016). We therefore believe that we have captured the majority of the root biomass with our plant-soil cores. All pots were immediately transported to a greenhouse (12 h of supplemental light of ~1 mmol m^{-2} s^{-1} using LED light, 30/20 °C during day/night, relative humidity: 60%) and clipped to 10 mm above the surface at the same day. The first shoot regrowth was initiated after bringing all soils to 65% water-holding capacity (WHC, corresponding to 32% gravimetric soil moisture content according to Mariotte *et al.*, 2020) with half of the pots watered once with a NH_4NO_3 solution at a rate of 10 g N m^{-2} and the other half with Milli-Q water. Soil moisture level was maintained at 65% WHC by weighing and watering pots daily to target weights. After 10 days of growth, half of the pots (*i.e.* half of each N treatment) were exposed to water-deficit conditions by suspending watering until soil moisture dropped to 40% WHC, while the other half was well-watered by keeping at 65% WHC. We employed a full factorial design, with one replicate pot from each treatment appearing in each of five blocks. The watering treatment conditions were maintained for the rest of the experiment. We are aware that keeping soil moisture at 40% WHC with high-frequency deficit irrigation (HFDI) may not be the same as drought in field conditions, as the added water will only rewet the top-soil layer where most of the roots are (Turner, 2019). Therefore, our HFDI treatment effects may not directly translate to drought effects in the field.

2.2. Plant N and P uptake

After 14 days, all plants were clipped immediately prior to ^{15}N and ^{32}P labelling to assess plant N and P uptake from soil during the second phase of shoot regrowth. With this clipping we mimicked herbivory in this moderately grazed grassland ecosystem. Approximate 50 g soil samples were taken from four spots with a corer (diameter of 10 mm, depth of 50 mm) within each pot right before pulse labelling for later analyses of plant-available soil N and P concentrations. A plastic straw was placed into each hole created after soil sampling to allow for labelled nutrient delivery as below. On the same day, pots were transported to the greenhouse facility of the Australian Nuclear Science and Technology Organization (ANSTO) in Lucas Heights, NSW, Australia (natural light condition, 30/20 °C during day/night, relative humidity: 50-85%). Each pot was pulse-labelled with a mixed solution containing 2 mg ^{15}N (99 atom%, half as $(^{15}NH_4)_2SO_4$ and half as $K^{15}NO_3$, Cambridge Isotope Laboratories Inc., Andover, MA, USA) and 1.0 MBq ^{32}P (0.2 µg ^{32}P as carrier-free H_3PO_4, Eckert & Ziegler product code 6032, reference date 6 Nov 2019) dissolved in 12 mL deionized water. The addition of H_3PO_4 may have acidified the soil locally, but given the very small amount added, we believe this effect to be negligible. The label solution for each pot was split in four doses of 3 mL and injected in the soil at 50 mm depth via four plastic straws (equally-spaced surrounding plant) that were previously inserted into the pot after soil sampling. Two weeks after labelling, shoot biomass was harvested by clipping to the soil surface and soil was subsampled after thoroughly mixing and excluding roots. Unfortunately, we were not able to harvest root biomass to measure root N and P concentrations in this study due to the high radiation hazard with collecting roots (Dijkstra et al., 2015; Mariotte et al., 2020).

Shoot biomass was dried in an oven at 60 °C for 24 h and weighed. Biomass samples were then clipped into small pieces (less than 5 mm length) and further ground with a coffee grinder for homogenisation. A subsample of 0.5 g homogenised shoot biomass was weighed into a crucible and ashed in a muffle furnace at 500 °C for 2 h. Ashed samples were mixed with 10 mL of concentrated HCl (12 M) and evaporated on a hotplate to 1-2 mL. After filtration (<0.45 µm), the solution was diluted to 50 mL with Milli-Q water and a 20 mL aliquot was analysed for ^{32}P activity by Cerenkov counting (L′Annuziata 2012) using a Packard Liquid Scintillation Analyser

(Tri-Carb 2900TR, PerkinElmer, Waltham, MA, USA, 5 min count time, 0-30 keV counting window). ^{32}P count rates were corrected for colour and chemical quench effects using matrix matched (*e.g.*, non-exposed shoots ashed and digested as per samples) quench standards and calculated ^{32}P activities were corrected for radioactive decay back to the ^{32}P reference date. At the beginning and the end of labelling after two weeks, plant-available soil P concentration was extracted with a 40 mL mixture of 0.03 M NH_4F and 0.025 M HCl from 3 g soil and shaken for 15 min at room temperature. Phosphorus concentration in the soil extracts was measured colourimetrically at 660 nm using the colouring reagent of ammonium paramolybdate-stanous chloride (Olsen & Sommers, 1982). For soil extracts collected two weeks after labelling, ^{32}P activity was determined according to the same protocol as that of plant biomass. Plant-available N concentration in soils collected at the beginning and the end of labelling was extracted with 1 M KCl and analysed colourimetrically using a flow-injection analyser (QuickChem FIA+, Lachat Instruments, Loveland, CO, USA). The ^{15}N of plant-available soil N was measured by adding MgO and Devarda's Alloy to the KCl extracts and trapping the NH_4^+ and NO_3^- using two filter paper disks acidified with 2.5 M $KHSO_4$ inside PTFE diffusion traps for 6 days (Stark & Hart, 1996). The filter paper disks were dried in a desiccator with concentrated H_2SO_4 and analysed for ^{15}N on a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility. Air-dried plant and soil samples were ball-milled and analysed for total N and ^{15}N on a Delta V Advantage isotope ratio mass spectrometer coupled to FlashHT and Conflo IV peripherals (Thermo-Fisher Scientific, Bremen, Germany). Unlabelled plants and soils were measured for ^{15}N signatures on the samples taken before pulse labelling.

Plant N and P uptake were determined for the whole community. For N uptake (U_N in mg N pot^{-1}, as NH_4^+ and NO_3^-) we used the following two-source mixing model (modified from Dijkstra et al., 2015):

$$U_N = N_{regrowth} \times (A_{15Nregrowth} - A_{15Nshoot})/(E_{15Nsoil} - A_{15Nshoot}) \qquad (1)$$

where $N_{regrowth}$ is the total amount of N in shoot regrowth (in mg N pot^{-1}), $A_{15Nregrowth}$ is the ^{15}N atom% in shoot regrowth, $A_{15Nshoot}$ is the weighted average ^{15}N atom% of shoots measured before labelling, and $E_{15Nsoil}$ is the ^{15}N enrichment factor of the plant-available soil N (Stark, 2000). Because $E_{15Nsoil}$ can decrease with time due to N mineralization, we estimated $E_{15Nsoil}$ with (Dijkstra et al., 2015):

$$E_{15Nsoil} = (A_{15Navail\ N\text{-}start} + A_{15Navail\ N\text{-}end})/2 \qquad (2)$$

where $A_{15Navail\ N\text{-}start}$ and $A_{15Navail\ N\text{-}end}$ are the ^{15}N atom% of the plant-available soil N at the start and end of ^{15}N labelling, respectively. For $A_{15Navail\ N\text{-}end}$ we used the ^{15}N atom% of NH_4^+ and NO_3^- measured in the soil extracts at the end of labelling, while $A_{15Navail\ N\text{-}start}$ was based on the amount of ^{15}N tracer added and background plant-available soil ^{15}N concentrations:

$$A_{15Navail\ N\text{-}start} = [N_{tracer} \times A_{15Ntracer} + N_{background} \times A_{15Nbackground}] / (N_{tracer} + N_{background}) \qquad (3)$$

where N_{tracer} and $A_{15Ntracer}$ are the amount (mg pot^{-1}) and ^{15}N atom% of the tracer, respectively, and $N_{background}$ and $A_{15Nbackground}$ are the available N (sum of NH_4^+ and NO_3^-, mg pot^{-1}) and its ^{15}N atom% measured directly before labelling. With these calculations we assumed that N uptake by the plant community would be in the form of NH_4^+ and NO_3^- and that uptake of organic forms of N would be negligible. Although plants take up dissolved organic N directly from soils (*e.g.*, Näsholm et al. 2009), this N uptake pathway was suggested to be of minor importance for N acquisition by most plants (Jones et al., 2005). Remineralization of ^{15}N-labelled organic matter from plant biomass turnover was not accounted since it is not very likely that actively growing plants taking up ^{15}N tracers would die during the two-week period of regrowth.

We calculated P uptake (U_P, in mg P pot^{-1}) by dividing the ^{32}P activity in shoot biomass ($A_{32Pregrowth}$, in Bq pot^{-1}) by the ^{32}P enrichment in the extractable soil P ($E_{32Psoil}$, in Bq mg^{-1} extractable P) at the end of the experiment (Dijkstra et al., 2015):

$$U_P = A_{32Pregrowth} / E_{32Psoil} \qquad (4)$$

We assumed that P mineralization during the two-week labelling period was small, and therefore, $E_{32Psoil}$ was estimated as the ^{32}P activity in the extractable soil pool at the end of the experiment. All ^{32}P activities were corrected for radioactive decay back to the ^{32}P reference date. We calculated re-allocation of N and P from roots to shoot regrowth of the whole plant community at the end of the two-week labelling period as the difference between total N and P in shoot regrowth and uptake of N and P, respectively.

2.3 Statistical analyses

Data were tested for normality using the Kolmogorov-Smirnov test and for homogeneity of variance using Levene's test. We used linear mixed models to examine the main and interactive fixed effects of HFDI and N addition and a random effect of treatment block (nested within HFDI and N addition) on plant-available soil N and P concentrations, plant biomass, plant N and P uptake, plant N and P re-allocation, and N:P ratios in shoot regrowth, of nutrients taken up, and of re-allocated nutrients. Duncan's multiple range tests were used to compare the means of each treatment combination. Pearson correlation analysis was employed to determine relationships among plant nutrient uptake, nutrient re-allocation and shoot regrowth. All statistical analyses were carried out using SPSS 16.0 (SPSS Inc., Chicago, USA) and statistical significance was set as $P < 0.05$.

3. Results

3.1 Shoot biomass and soil N and P availabilities

Shoot biomass was highly enriched in ^{15}N and ^{32}P after pulse labelling (Table S1), indicating that the pulse labelling of plants was successful. Nitrogen addition increased shoot biomass, both in the first and second regrowth, while HFDI showed the opposite effect (Fig. 1a,b; Table S2).

Directly before labelling, *i.e.* one month after N addition and at the start of the second regrowth stage, plant-available soil N concentration was not different between control and N addition treatments under well-watered condition (Table 1). Plant-available soil N concentration increased with N addition with HFDI (a significant HFDI × N interaction) and was higher than in other treatments both at the beginning and at the end of the second regrowth (all $P < 0.001$, Table 1). Plant-available soil P concentration marginally increased with HFDI ($P = 0.09$) at the beginning of the second regrowth but tended to decrease with N addition for the two sampling times (Table 1). Consequently, ratios of plant-available soil N:P were highest under combined HFDI and N addition ($P < 0.001$, Table 1).

3.2 Plant N and P uptake and re-allocation

Shoot N concentration increased with N addition ($P = 0.009$), while shoot P concentration decreased with N addition ($P = 0.015$) and increased with HFDI ($P < 0.001$, Table 2). Plant N uptake increased with N addition only with HFDI, showing a significant HFDI × N interaction and the highest uptake under combined HFDI and N addition ($P < 0.001$, Fig. 2a). In contrast, plant P uptake showed no response to both HFDI and N addition (Fig. 2b). Re-allocation of both plant N and P increased with N addition under well-watered condition but decreased with HFDI under N addition, resulting in significant HFDI × N interactions (*P* = 0.002 for N and 0.003 for P; Fig. 2a,b). Re-allocation accounted for 48-97% of total N and 58-79% of total P in shoot regrowth across HFDI and N addition treatments (Fig. S1).

3.4 Stoichiometric N:P ratios in shoot regrowth, uptake and re-allocation, and correlation analyses

Nitrogen addition increased N:P ratios in shoot regrowth ($P < 0.001$) and of re-allocated nutrients ($P = 0.04$, Table 2). The N:P ratio of nutrient uptake was highest in combined HFDI and N-addition treatments, resulting in a marginally significant main effect of HFDI and interactive effect of HFDI and N addition (Table 2).

Nitrogen re-allocation was positively correlated with P re-allocation (Fig. 3a) with both nutrients positively contributing to shoot regrowth (Fig. 3b,c). In contrast, shoot regrowth, plant N uptake and plant P uptake showed no relationships with each other (Fig. S2).

4. Discussion

While numerous isotope pulse-chase studies have been conducted, very limited progress has been made in quantifying the relative contribution of plant nutrient uptake from soils and nutrient re-allocation from roots to shoots (Clark, 1977; Dijkstra et al., 2018). In this study, we used a novel isotopic dual-labelling approach (^{15}N and ^{32}P) to assess the reliance of grassland plant shoot regrowth on uptake of external N and P from soils *vs.* internal re-allocation of N and P from roots. Nitrogen and P uptake and re-allocation from roots to shoot regrowth were quantified from intact plant-soil cores taken from a grassland, and after clipping the plants to simulate herbivory. We found that re-allocation of N and P instead of uptake were tightly coupled with each other. Additionally, nutrient re-allocation contributed more significantly to new shoot regrowth during the first two weeks than that of nutrient uptake. Our findings demonstrate the importance of re-allocation of nutrients from roots in maintaining a balanced supply of N and P for plant regrowth to buffer against fluctuations in availability of N and P in the soil. This process of nutrient re-allocation from roots is particularly important for recovery of plant productivity in grasslands that are being grazed.

4.1 Effects of HFDI on N and P uptake and re-allocation without N addition

Contrary to our expectation, HFDI had no impact on uptake of N and P without N addition. A lack of a HFDI effect on N uptake could be mainly attributed to similar levels in soil N availability between well-watered and HFDI treatments without N addition (Table 1). Treatment of HFDI caused a non-significant increase in soil P availability (Table 1), but uptake of P was still unaffected by HFDI without N addition (Fig. 2b). Possibly, drier soils with HFDI resulted in a greater diffusion barrier of free PO_4^{3-} (He & Dijkstra, 2014) counteracting higher concentrations of plant-available soil P with no net effect on P uptake. Non-significant HFDI effects on shoot biomass without N addition during the second regrowth (Fig. 1b) further confirmed that soil availability and plant accessibility of nutrients might show a stronger effect on plant growth than that of water availability under HFDI, although this would also depend on the magnitude and duration of water deficit (Gessler et al., 2017; Schönbeck et al., 2020). A greater magnitude and longer duration of water deficit may override the effects of soil nutrient availability in reducing plant productivity and nutrient re-allocation from roots (Schönbeck et al., 2020). Under these conditions hydraulic failure and carbon starvation can irreversibly impair plant physiological functions causing root damage and mortality (Gessler et al., 2017).

Without N addition, N and P in shoot regrowth were largely supplied via internal re-allocation (94% and 69%, respectively, averaging across watering treatments; Fig. S2), suggesting that internal recycling of nutrients is the main nutrient-supply pathway over soil nutrient uptake for biomass production under low nutrient availability, at least during the first two weeks of regrowth. Without N addition, HFDI had no effect on re-allocation of N and P. This is in contrast to a global assessment of N and P resorption from leaves before senescence, which increased for N but decreased for P with decreasing water availability (Yuan et al., 2009). While the direction and magnitude of nutrient resorption are also regulated by other factors, such as soil nutrient availability, the size of nutrient reserves in plant storage organs, and C investment for mobilising nutrients (Yuan et al., 2009; Masclaux-Daubresse et al., 2010), nutrient resorption from senesced leaves may not equate to nutrient re-allocation in grazed grasslands. We clipped plants when shoots were still green and therefore without or little nutrient resorption occurring prior to regrowth. Under these conditions, re-allocation of nutrients to shoots will depend on the capacity of plants to store nutrients in roots directly derived from soil rather than from leaf resorption. Perennial grass species, especially the rhizomatous species, generally have a high capacity of nutrient reserves in roots that facilitate plant regeneration and resistance to nutrient fluctuations and grazing pressures (Klimešová et al., 2018; Pereira-Silva et al., 2019).

4.2 Effects of HFDI on N and P uptake and re-allocation with N addition

Unexpectedly, HFDI increased plant N uptake with N addition (Fig. 2a & 4), which contrasted with our previous assumption that HFDI decreases soil N availability and plant N uptake from soils. During the first regrowth, possibly much less of the added N was taken up by plants under HFDI than under well-watered conditions, causing accumulation of plant-available soil N after N addition under HFDI (Table 1). As a result, during the second regrowth, plants under HFDI had greater access to soil N, thereby increasing N uptake compared with plants under well-watered condition. Correspondingly, a decrease in re-allocation of N was detected under HFDI with N addition (Fig. 2a), suggesting a shift in strategies of plant N acquisition from re-allocation to uptake when N becomes abundant with HFDI (Eyles et al., 2009; Dijkstra et al., 2018). Presumably, during the first regrowth, a reduction in growth as induced by HFDI (Fig. 1a) reduced N uptake, but may have increased re-allocation of N when plants still had enough reserves of N in roots to support shoot regrowth under HFDI conditions (Fig. 4; Milla et al., 2005). Consistently, we found significant increases in both root biomass and root N concentration (*i.e.* higher root N reserve) under HFDI with N addition during the first regrowth in a parallel study (Wang et al., 2021a). During the second regrowth, N reserves in roots were likely lower, and plants switched back to taking up N from the soil, as soil N supply was now larger (Table 1 & Fig. 4). Unfortunately, we were not able to measure root N content (or N reserves) during the two stages of regrowth in this study due to the high radiation hazard of the root biomass, which could have corroborated this. Nevertheless, out results suggest that these grassland plants may maintain a dynamic balance between N uptake and re-allocation that depend on the relative access and associated expense of using both N sources for regrowth (Fig. 4; Dijkstra et al., 2018).

Inconsistent with our first hypothesis, plant P uptake remained unchanged in response to HFDI with N addition, which was remarkably different from that of N uptake. We expected stronger negative effects of HFDI on soil P diffusion, mass flow and transport from soils to plants than that of N (Fig. 4; Lambers et al., 2008; He & Dijkstra, 2014). However, as was argued above, during the second regrowth, plants may have been more limited by P than by water, so that HFDI had no effects on P uptake. Interestingly, in a companion study we observed that HFDI reduced colonisation of arbuscular mycorrhizal fungi in this grassland community (Wang et al., 2021b), suggesting that plants relied less on mycorrhizal symbionts to take up nutrients under these conditions. Arbuscular mycorrhizal fungi tend to be particularly important for P uptake (Smith & Smith, 2011), but apparently, a potential reduction in mycorrhizal colonization had no effect on plant P uptake. This HFDI-induced reduction in mycorrhizal colonisation contradicts with the frequent observation that arbuscular mycorrhizal fungi benefit plants for taking up nutrients under drought (*e.g.*, Mariotte et al., 2017). The discrepancy might be due to the fact that HFDI, unlike drought, results in an uneven distribution of water in the soil consequentially causing distinct effects as compared with drought (Turner, 2019). With N addition, HFDI decreased re-allocation of P, similar to the reduction in N re-allocation. Possibly, HFDI decreased hydrolysis of nutrient-storage compounds and transport of remobilised nutrients due to desiccation of plant roots and disruption in the plant water flow (Estiarte & Peñuelas, 2015). Moreover, our results suggest a coupled remobilisation of N and P from compounds presented in roots (*e.g.*, proteins, amino acids, nucleic acids) (Cooke & Weih 2005; Chapin & Jones, 2009). Overall, the contrasting responses of N and P uptake and re-allocation to HFDI indicate that these two processes of plant nutrient acquisition for new growth respond to an interplay of soil water and nutrient availability with the effects of HFDI strongly affected by soil N supply (Weatherall et al., 2006; Dijkstra et al., 2018).

4.3 Stoichiometric N:P ratios of uptake and re-allocation

With N addition, HFDI remarkably increased plant N uptake, but not P uptake resulting in higher stoichiometric plant N:P uptake ratios (Table 2), as we hypothesized. This can be attributed to the synergistic effects of HFDI and N addition on plant-available soil N accumulation (Yue et al., 2019) causing much higher plant-available soil N:P ratios under combined HFDI and N addition (Table 1). Also, the variability in N:P ratios measured in shoot regrowth was much smaller compared with the variability in N:P ratios from soil uptake (Table 2). Given that a large proportion of N and P in shoot regrowth was derived from roots and not from soil (particularly without N addition, Fig. 4), our results indicate that plants have the capacity to maintain a balanced supply of N and P to support new shoot regrowth (Riley et al., 2019), to buffer against changes in availability of N and P in the soil caused by HFDI and N addition. Overall, our results highlight the need to consider re-allocation of N and P from roots as an important mechanism for plants to maintain N:P stoichiometry in grazed pastures affected by environmental change (Vizoso et al., 2008; Dijkstra et al., 2018).

While HFDI and N addition resulted in the highest N:P ratio in shoot regrowth derived from soil uptake, supporting the second part of our third hypothesis, the N:P ratio derived from re-allocation only increased with N addition (Table 2). Possibly, with N addition, plants were able to store more N than P before the second regrowth occurred, thereby supporting the second regrowth with relatively more N than P from re-allocation (Thornton et al., 1994; Riley et al., 2019). Relatively higher re-allocation of N than P with N addition could also indicate that plant regrowth became more limited by P. The N:P ratios measured in shoot regrowth were around 16, a cut-off value that has frequently been used to indicate P limitation in plants (Güsewell, 2004). Regardless, our results show that whether plant growth was limited by N or P not only depending on availability of N and P in the soil, but also on N and P reserves in roots.

5. Conclusions

Our novel ^{15}N and ^{32}P tracer method demonstrates that re-allocation of N and P from roots is an important strategy of conservative nutrient use that supports new plant growth in water-deficit conditions. Uptake and re-allocation of both N and P were not affected by HFDI without N addition, because during the second regrowth, plant-available soil N and P concentrations were low, and plants relied predominantly on re-allocation of N (94%) and P (69%) to support shoot regrowth. With N addition, HFDI unexpectedly enhanced plant N uptake, likely due to accumulation of plant-available soil N from exogenous N supply in water-deficit conditions. However, re-allocation of root N and P decreased under HFDI with N addition, presumably because nutrient re-allocation became more difficult than uptake from the soil. Our results further suggest a coupled remobilisation of N and P from compounds presented in roots. Smaller variability in N:P ratios of shoot regrowth than that of from soil uptake revealed the pivotal role of root nutrient re-allocation in maintaining balanced plant nutrient supply and buffering against changes in soil N and P availability under HFDI and N-enrichment conditions. It is possible that severe water deficit may result in different responses of plant nutrient uptake and re-allocation due to irreversibly root damage and mortality. Our study clearly reveals that re-allocation of nutrients from plant roots is an essential plant trait supporting shoot regrowth and should be considered in biogeochemical N and P cycling models to better predict ecosystem productivity.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

F.A.D. conceived the research; R.W., F.A.D., T.C., M.P.J., J.J.H., and C.K. performed the research including isotopic and elemental measurements; all author participated in the evaluation and interpretation of the results; R.W. analysed the data and drafted the manuscript, and all authors, especially Y.J., C.K., T.R.C., and F.A.D. contributed to further revising of the text. All authors read and approved the manuscript.

Data availability: Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.dbrv15f21 (Wang et al., 2021).

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Tables

Table 1 Plant-available soil nitrogen (N) and phosphorus (P) concentrations directly before labelling during the second regrowth (Start) and two weeks after labelling (End). Data are means ± standard error (*n* = 5) and different letters show significant differences among treatments within each sampling time (T). *P* values are reported below each parameter for ANOVAs conducted across treatment combinations for each sampling time. N0 and N+: 0 and 10 g N m^{-2}; HFDI: high-frequency deficit irrigation treatment with 40% water holding capacity.

	Available N (mg kg^{-1})		Available P (mg kg^{-1})		Soil N:P	
	Start	End	Start	End	Start	End
N0	3.4 ± 0.4b	5.5 ± 0.6b	6.8 ± 1.7ab	5.9 ± 2.1a	0.62 ± 0.17b	1.3 ± 0.29b
N+	6.6 ± 1.4b	10.9 ± 2.7b	5.3 ± 0.8b	3.0 ± 0.7a	1.3 ± 0.22b	3.9 ± 1.1b
HFDIN0	5.3 ± 1.2b	7.5 ± 0.8b	14.4 ± 4.1a	8.9 ± 2.4a	0.48 ± 0.15b	1.0 ± 0.20b
HDFIN+	44.2 ± 5.9a	72.4 ± 8.9a	7.0 ± 1.6ab	5.8 ± 1.5a	7.1 ± 1.4a	15.8 ± 3.2a
ANOVA *P*-values						
HFDI	< 0.001	< 0.001	0.09	0.16	0.002	0.008
N	< 0.001	< 0.001	0.10	0.15	< 0.001	< 0.001
HFDI×N	< 0.001	< 0.001	0.27	0.95	0.002	0.006

Table 2 Shoot nitrogen (N, mg g^{-1}) and phosphorus (P, mg g^{-1}) concentrations, and N:P ratios in shoot regrowth, of nutrients taken up, and of re-allocated nutrients from roots to shoot regrowth under high-frequency deficit irrigation (HFDI) and N addition. Results are from second regrowth. *P* values for ANOVAs are reported below each parameter. N0 and N+: 0 and 10 g N m^{-2}; HFDI: treatment with 40% water-holding capacity.

	Shoot N	Shoot P	Shoot N:P	Uptake N:P	Recycle N:P
N0	19.4 ± 0.4b	2.0 ± 0.3b	10.3 ± 1.5b	1.8 ± 0.5b	13.5 ± 1.4ab
N+	19.1 ± 1.3b	1.2 ± 0.1c	15.7 ± 1.5a	2.7 ± 0.6b	19.3 ± 1.8a
HFDIN0	20.8 ± 0.9b	2.7 ± 0.2a	7.7 ± 0.3b	2.4 ± 0.6b	11.1 ± 1.1b
HFDIN+	24.9 ± 1.3a	1.6 ± 0.3bc	16.3 ± 1.6a	29.5 ± 13.7a	15.0 ± 4.0ab
ANOVA *P*-values					
HFDI	0.12	<0.001	0.41	0.07	0.14
N	0.009	0.015	<0.01	0.07	0.04
HFDI × N	0.70	0.48	0.19	0.08	0.66

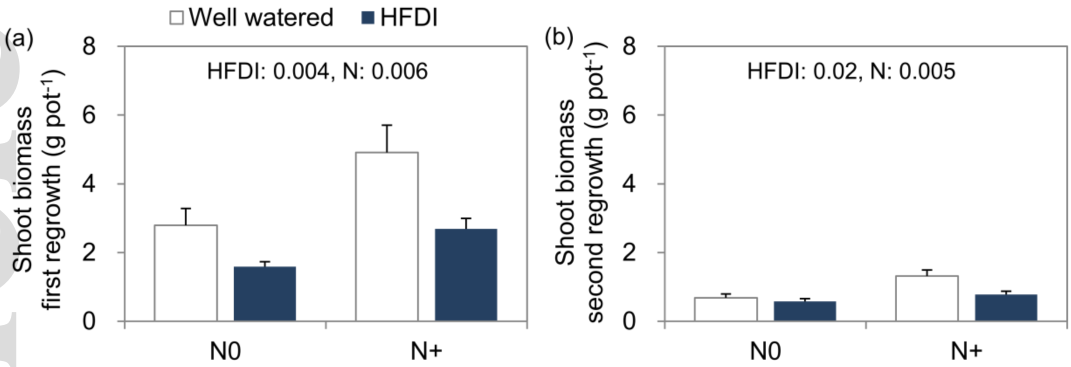
Figure captions

Figure 1 Shoot biomass clipped directly before labelling (first regrowth, a) and two weeks after labelling (second regrowth, b) as affected by high-frequency deficit irrigation (HFDI) and nitrogen (N) addition. Error bars represent standard error (*n* = 5) and *P* values for ANOVA are reported when significant ($P < 0.05$). N0 and N+: 0 and 10 g N m^{-2}; well watered and HFDI: 65 and 40% water holding capacity.

Figure 2 Plant uptake and re-allocation of nitrogen (N) (mg pot^{-1}, a) and phosphorus (P) (mg pot^{-1}, b) under high-frequency deficit irrigation (HFDI) and N addition. Error bars represent standard error (*n* = 5) and different letters above error bars show significant differences among treatments for nutrient uptake (lowercase letters) and re-allocation (capital letters) separately. Results are from second regrowth. *P* values for ANOVAs are reported when significant ($P < 0.05$). N0 and N+: 0 and 10 g N m^{-2}; well watered and HFDI: 65 and 40% water holding capacity.

Figure 3 Relationships between nitrogen (N) re-allocation and phosphorus (P) re-allocation (a), between N re-allocation and shoot regrowth (b), and between P re-allocation and shoot regrowth (c). Results are from second regrowth. N0 and N+: 0 and 10 g N m^{-2} respectively; non-drought and drought: 65 and 40% water holding capacity respectively. Unfilled circles and triangles represent N0 and N+ with well-watered treatment, respectively; filled circles and triangles represent N0 and N+ with high-frequency deficit irrigation treatment, respectively. The grey shading represents 95% confidence intervals.

Figure 4 Nitrogen (N) addition under well-watered conditions increases the size of the root N pool prior to clipping, and thus promotes re-allocation of N from roots coupled with higher P re-allocation to support shoot regrowth after clipping. High-frequency deficit irrigation (HFDI) slows down uptake of added N and storage of N in roots prior to clipping, resulting in accumulation of plant-available soil N. Consequently, under HFDI, plants can rely less on re-allocation but rely more on soil N uptake to support shoot regrowth. Even with relatively high soil P availability under HFDI conditions, plant P uptake remains unchanged possibly due to slower P diffusion in dry soils. Pie figures show proportional N and P from soil uptake and from re-allocation with N addition under well-watered and HFDI conditions.



□ Well watered
■ HFDI
(a)
Shoot biomass first regrowth (g pot^{-1})
8
6
4
2
0
HFDI: 0.004, N: 0.006
N0
N+
(b)
Shoot biomass second regrowth (g pot^{-1})
8
6
4
2
0
HFDI: 0.02, N: 0.005
N0
N+

Figure 1

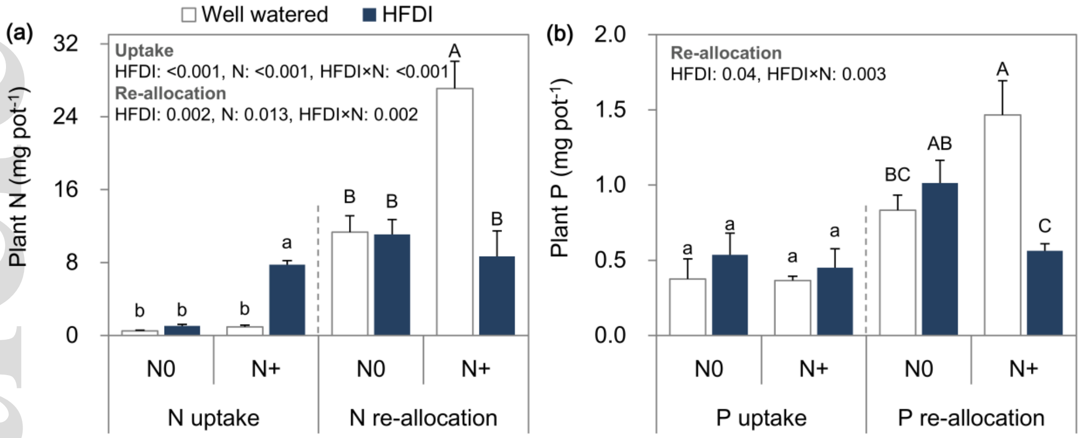

□ Well watered
■ HFDI
(a)
Plant N (mg pot^{-1})
32
24
16
8
0
Uptake
HFDI: <0.001, N: <0.001, HFDI×N: <0.001
Re-allocation
HFDI: 0.002, N: 0.013, HFDI×N: 0.002
b
b
b
a
B
B
A
B
N0
N+
N0
N+
N uptake
N re-allocation
(b)
Plant P (mg pot^{-1})
2.0
1.5
1.0
0.5
0.0
Re-allocation
HFDI: 0.04, HFDI×N: 0.003
a
a
a
a
BC
AB
A
C
N0
N+
N0
N+
P uptake
P re-allocation

Figure 2

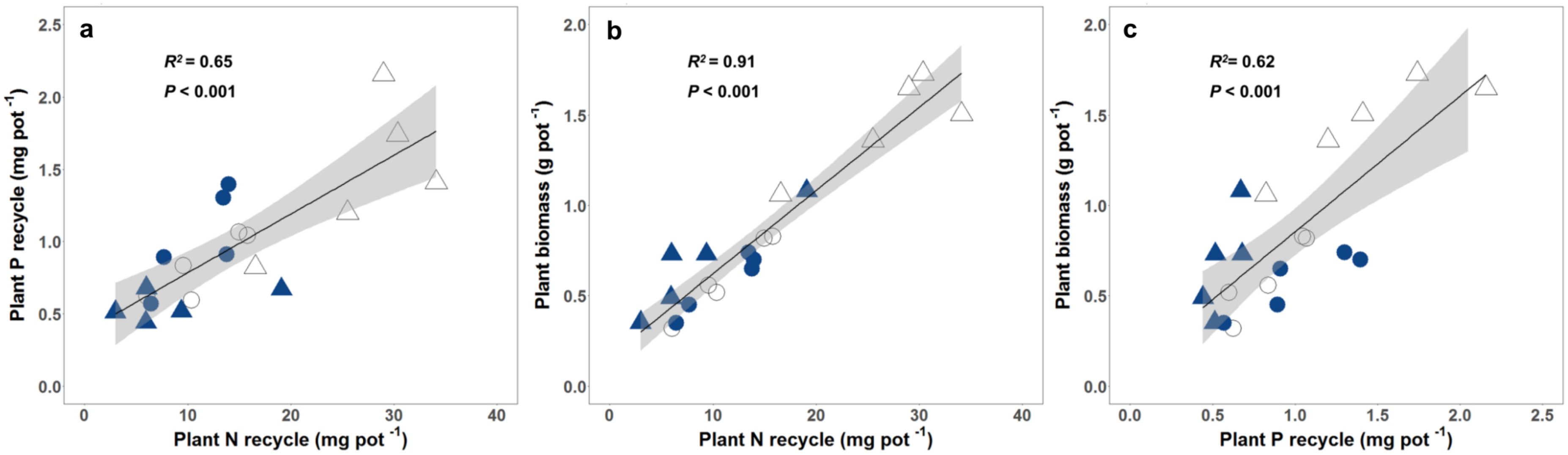

a
$R^2 = 0.65$
$P < 0.001$
Plant P recycle (mg pot $^{-1}$)
Plant N recycle (mg pot $^{-1}$)
0.0
0.5
1.0
1.5
2.0
2.5
0
10
20
30
40
b
$R^2 = 0.91$
$P < 0.001$
Plant biomass (g pot $^{-1}$)
Plant N recycle (mg pot $^{-1}$)
0.0
0.5
1.0
1.5
2.0
0
10
20
30
40
c
$R^2 = 0.62$
$P < 0.001$
Plant biomass (g pot $^{-1}$)
Plant P recycle (mg pot $^{-1}$)
0.0
0.5
1.0
1.5
2.0
0.0
0.5
1.0
1.5
2.0
2.5

Figure 3

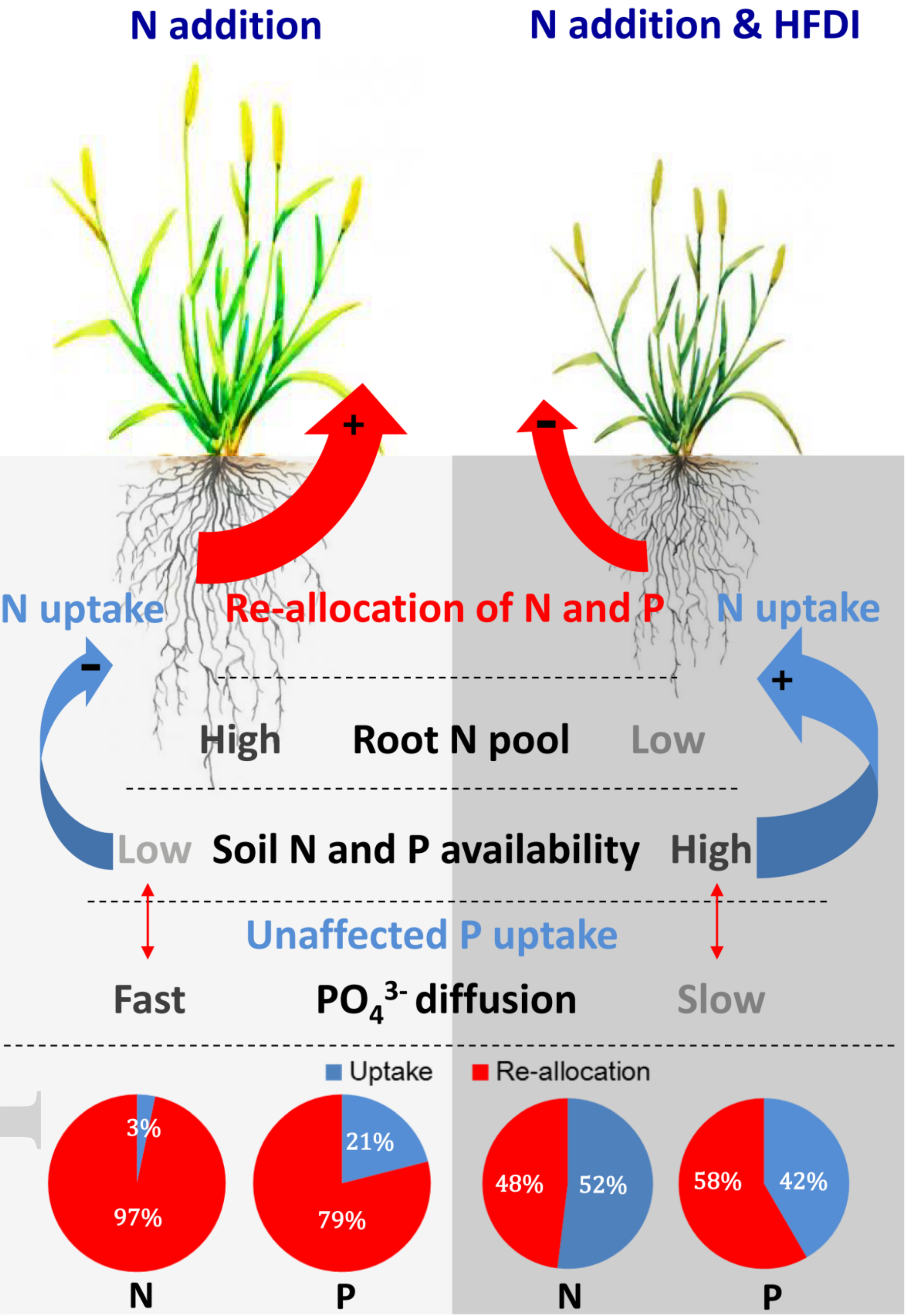

N addition
N addition & HFDI
+
−
N uptake
Re-allocation of N and P
N uptake
−
+
High
Root N pool
Low
Low
Soil N and P availability
High
Unaffected P uptake
Fast
PO_4^{3-} diffusion
Slow
🟦 Uptake
🟥 Re-allocation
3%
97%
21%
79%
48%
52%
58%
42%
N
P
N
P

Figure 4