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RESEARCH ARTICLE

Using microdialysis to assess soil diffusive P and translocated sap flow P concentrations in Southern *Pinus taeda* plantations

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Abstract

Aims To improve soil phosphorus (P) testing in silvicultural systems, we assess microdialysis to study concentrations and establish a standard methodology to assess soil diffusive P and in-vivo translocated sap flow P under variable rates of P carryover from a previous rotation across various soils.

Methods Soils were collected from each treatment in the field and analyzed in laboratory conditions. Soils were analyzed for diffusive soil P using microdialysis and Mehlich III for comparison. Sap flow P measurements were collected in the field from 16 trees, one tree per treatment and replication over four hours.

Results Spodosol soils had higher diffusive P levels than Alfisol soils. On average, diffusive P increased by 137% in Spodosol and 166% in Alfisol from pre- to post-planting of a new stand. In the Alfisol, diffusive P showed a strong relationship with tree height, while no significant association was observed in the Spodosol. The Mehlich III soil extractions were positively related to the Alfisol but not the Spodosol. Microdialysis samples collected from the trees responded to changes in fertilization rates and were shown to be positively related to tree heights and Mehlich soil P tests. Atmospheric conditions substantially impacted sap flow P, with samples collected in full sunlight showing an average increase of 100% compared to overcast conditions.

Conclusions These findings demonstrate the potential of microdialysis as a valuable tool for soil P testing and its application in addressing complex questions related to P translocation and tree physiology in silvicultural settings.

Keywords Microdialysis · Translocated phosphorus · Diffusive phosphorus · *Pinus taeda* · Phosphorus fertilization

Introduction

The availability of phosphorus (P) is subject to a complex interplay of chemical and physical environmental factors, which collectively determine its accessibility to plants. While P is crucial for plant growth, it is also the least mobile and readily available macronutrient, making it one of the most common limiting nutrients after nitrogen (N) (Raghothama and Karthikeyan 2005). P deficiencies are prevalent in regions like the Atlantic Coastal Plain, which is characterized by highly weathered acidic soils with minimal native exchangeable P left in the underlying parent material (Polglase et al. 1992).

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Plants have evolved various strategies to acquire P, including increasing their total root surface through both macroscopic and microscopic root growth (Jungk 2001). They also release extracellular enzymes known as phosphatases and phytases to extract P from organic sources and establish partnerships with mycorrhizal fungi, which enhance these root-based strategies (George et al. 1995). These mining strategies create zones of P depletion around the roots and extend deeper into the soil through the mycorrhizal extraradical mycelium emanating from the fungi (Bhat and Nye 1973). The strength of this diffusional gradient and its impact on the desorption of P from soil surfaces are influenced by the initial soil P content, as well as the soil's chemical and morphological characteristics (Holford 1997). Therefore, for a comprehensive understanding of how plants take up P from their surroundings, there is a need to develop methods that can model this concentration gradient with greater precision (Santner et al. 2012).

Natural sources of P and fertilization

Understanding the relationships between soil chemical properties and long-term phosphorus (P) availability is crucial for predicting the response to P fertilization. In phosphorus P-limited environments where the soil's natural supply falls short of plant requirements, fertilization is employed to compensate for the deficiency. For Pinus taeda L. stands situated on the Atlantic Coastal Plain, studies have demonstrated the positive effects of P fertilization in enhancing growth on P-deficient soils (Pritchett and Swinford 1961; Gent et al. 1986). Consequently, this practice has become commonplace in plantation management (Fox et al. 2011). While the build-up of P from fertilization in mineral soils has been extensively examined in the realm of agriculture, it remains a relatively poorly understood phenomenon in forest systems (Zhang et al. 2020; Zhu et al. 2018; Menezes-Blackburn et al. 2018).

It is worth noting that nearly 80% of the applied P in the form of fertilization is rapidly transformed into insoluble compounds, with the remaining P existing in solution or being taken up by the trees for immediate utilization (Holford 1997). Intriguingly, research has revealed that a single P application on these deficient sites can sustain optimal productivity for Pinus taeda throughout the entire duration of a stand rotation and even into the subsequent rotation, thanks to the presence of residual P (Pritchett and Comerford 1982; Comerford and De Barros 2005; Everett and Palm-Leis 2009). This residual P, which lingers in the stand from the forest floor and subsoil as a result of previous fertilization and its availability for the subsequent rotation, is often referred to as the P carryover effect or legacy effect (Everett and Palm-Leis 2009).

In coniferous forest ecosystems, P is either retranslocated into fresh tissues or lost through litter fall and the ongoing decomposition of leaf litter (Fox et al. 2011). This phenomenon essentially means that mature trees in established stands serve as a significant source of P for themselves. However, once these m ature trees are harvested, the mineral soil is left incapable of replenishing P due to the absence of P-containing minerals in the parent material. During the early stages of a new stand, young trees rely solely on any residual build-up that might have occurred due to previous P fertilization and the decomposition of organic matter. These results suggest that a substantial portion of the P in these soils comes from the mineralization of organic materials and the previous forest floor rather than the mineral soil itself.

The findings of the study by Everett and Palm-Leis (2009) represent the only research conducted in the southeastern region that investigated P carryover in pine plantations with known variable rates of P from a previous rotation, focusing on a specific poorly-drained albaquult soil type. We hypothesize that trees grown on other soil types with different soil chemistries and morphologies will maintain optimized P levels in their foliage for a more extended period due to the carryover of P from the previous rotation. This carryover could potentially reduce the need for early rotation P fertilization. Therefore, gaining a comprehensive understanding of how residual P fertilizers persist across multiple rotations on various soils and parent materials is crucial for optimizing early P fertilization practices throughout the southeastern United States.

Soil extraction methods

Plants absorb P as soluble inorganic orthophosphate ions in solution (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^{-}$) via diffusion by active membrane transporters on the root's surface, as well as through the of mycorrhizal fungi (Schachtman et al. 1998; Plassard et al. 2019). The

soluble phase of P without plant root interference can be viewed as the soil's equilibrium of P's dissolution, desorption, and mineralization in that soil at any given time (Stevenson & Cole 1999). The soluble phase P pool is the smallest, often containing less than 0.2 mg L^{-1} P even on highly fertile agricultural soils, implying that most plant-available P originates from the solid phases of soil P (Grant et al. 2005). Solution P can be immobilized via soil microbes or plants, absorbed onto mineral surfaces, or precipitated with secondary Fe and Al hydrous oxide compounds found in the soil (Schachtman et al. 1998). Depending on environmental and soil conditions, the remaining unavailable pools of P can be multiple orders of magnitude higher than solution P.

Methods such as Mehlich III (Mehlich 1984), resin-P (Sibbesen 1978), and fractionation (Hedley et al. 1982) separate or incorporate one or more P pools into a final reported value. These approaches, however, are often difficult to relate to soluble phase soil P because they also mobilize one or more of the unavailable forms of P that may not be available to plants. Mehlich III soil testing is widely used in the southeast because it is optimized for acidic soils to provide fertilizer recommendations (Mehlich 1984; Wells et al. 1986). Mehlich III strips inorganic and organic forms of P from the soil using a combination of acetic acid (CH_3COOH), ammonium nitrate (NH_4NO_3), ammonium fluoride (NH_4F), nitric acid (HNO_3), and ethylenediaminetetraacetic acid (EDTA) at pH 2.5 to determine both readily and slowly available P. The resulting solution is quantified colorimetrically or by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). This solution contains inorganic and organic forms of P that were stripped from the soil and dissolved into solution. Mehlich III is a valuable tool to test for acid-extractable forms of P; however, in highly P deficient soils, with < 6 mg kg^{-1} of extractable P, Mehlich III soil tests are difficult to correlate to increases in *P. taeda* productivity (Wells et al. 1986). This difficulty in establishing a relationship between Mehlich III and *P. taeda* on these soils highlights the need for an alternative soil test to better predict responses to growth and available P.

Microdialysis

Microdialysis bears many comparisons to a plant root (Demand et al. 2017; McKay Fletcher et al. 2021) and potentially offers researchers a relatively simple method to assess P uptake from diffusive gradients under various circumstances. Microdialysis emerged from neurobiology and pharmacokinetics to study the concentrations of neurotransmitters and compounds in both in vivo and in vitro solutions via passive diffusion (Kalant 1958; Plock and Kloft 2005). The process of microdialysis requires an infusion pump, a probe, a perfusate solution, and a collection tube or vial. The probe consists of a thin inner tube surrounded by an outer semi-permeable membrane, which pushes the perfusate through the membrane, allowing solutes to passively exchange across the surface into the perfusate solution depending on the concentration of gradients of both the probe and exterior solution. The microdialysis pump pushes the perfusate solution through the microdialysis probe at a constant push-pull rate ranging from 0.5 – 5 µl/min. The dialysate (perfusate that has passed through the probe) is collected in a small collection receptacle (Fig. 1). The result is that a dialysate solution mimics the concentrations of metabolites, compounds, and overall composition of the tissue or matrix being tested.

Microdialysis has made significant breakthroughs in studying environmental equilibrium samples by offering a minimally destructive method for assessing ion concentrations in solution. Microdialysis testing of soils has led to a greater understanding of diffusive N (Inselsbacher and Näsholm 2012), heavy metal concentrations in soil solutions (Mosetlha et al. 2007), and P availability in soil solutions (Demand et al. 2017; Petroselli et al. 2021). Equilibrium experiments are commonly used to measure the diffusion of solutes through the microdialysis probes. These equilibrium methods can be performed on inorganic or organic soil phases by adding a known amount of P into the soil and waiting for the soil to reach equilibrium before the solution is remeasured (Barrow 1978; Oburger et al. 2011). These experiments provide valuable information regarding P dynamics in bulk soil, similar to how a plant root takes up P from the soil. Microdialysis takes the root interactions into account by creating a pseudo-rhizosphere mimicking both the exudation of P mobilizing compounds, such as citrate, in a process called retrodialysis (Ryan et al. 2014) and the creation of the diffusional sink that occurs near the plant roots (Demand et al. 2017; Oburger et al. 2011; McKay Fletcher et al. 2021).

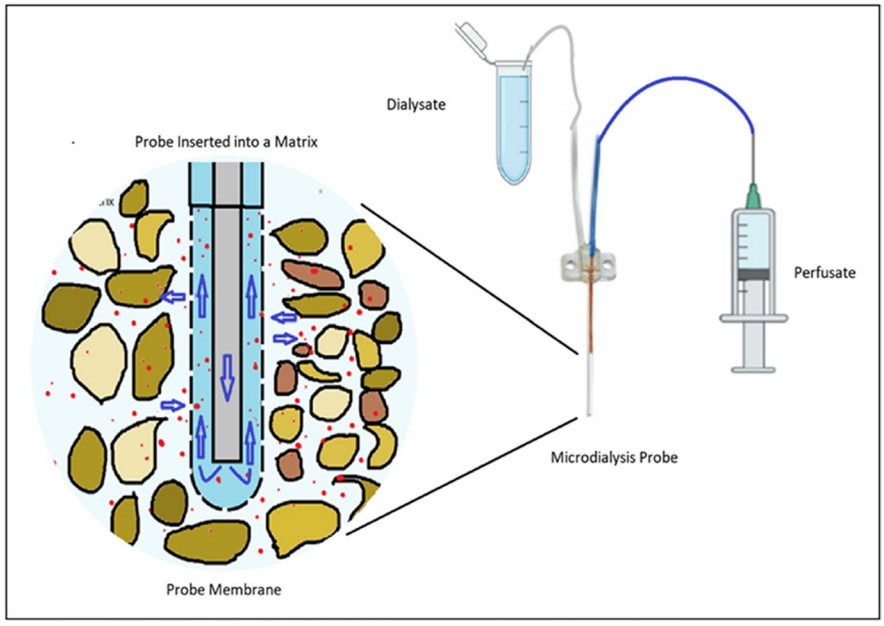

Fig. 1 Experimental setup of a microdialysis probe is inserted into a matrix; input into the probe is called the perfusate; the output collected is the dialysate. A syringe pump controls the pump rate. Image of microdialysis probe adapted from Harvard apparatus. Holliston, MA

The P that is mobilized and sampled by microdialysis over a given time could be analogous to the concentrations of P taken up by tree roots and provide an additional method for analyzing plant available quantities of nutrients (Shaw et al. 2014; Demand et al. 2017; Inselsbacher and Näsholm 2012). However, since microdialysis works in a microscale environment, scaling up the results is difficult due to microsite variations, including changes to soil chemistry, morphology, temperature, and moisture. These factors have been shown to influence solution P and, thus, the flux rates of P across the microdialysis membrane (Demand et al. 2017). Considering most soil P sampling done commercially is performed via multistep extraction procedures that extract available and unavailable forms of P, establishing a standardized method for soil sampling using microdialysis paired with these conventional soil sampling procedures will begin to reveal these relationships for various soils.

In addition to assessing P deficiencies in the soil using microdialysis, changes in P concentrations within the xylem sap of *P. taeda* have also been reported in P-deficient conditions (Sung et al. 2015; Lima et al. 2000; Mason et al. 2008). Current approaches to observe these changes in xylem sap composition require destructive sampling methods to extract these compounds from plant tissue. Microdialysis offers a method to sample metabolite concentrations within living plant tissue in a minimally destructive sampling method. To our knowledge, only two experiments have attempted to study in vivo plant metabolites: Pretti et al. (2014) used microdialysis to study ascorbic acids and antioxidants in water-stressed *Opuntia fixus indica*, and Jeřábek et al. (2020) was the first report to show high-frequency in situ measurements of P in the xylem sap of living beech trees using microdialysis probes. Diurnal fluctuations detected in these probes over 24 h were consistent with destructive sampling methods (Clark et al. 1986; Siebrecht et al. 2003), albeit at significantly lower overall concentrations in some cases, demonstrating this method is a viable option to track xylem P within the sap with the probes (Mason et al. 2008). Considering microdialysis probes are an effective method to track P in both soils (Demand et al. 2017) and arguably in beech trees (Jeřábek et al. 2020), we hypothesize that increased fertilization rates of P from a previous rotation will be represented as increases in the concentration of P collected from in-vivo dialysates of juvenile *P. taeda* trees.

Goal and objectives

Our main goal for this research was to explore the viability of microdialysis probes as an effective tool to assess P concentrations of diffusive P carryover

effects in southern pine plantations via an in-vitro soil equilibrium concentration experiment. Our objectives to achieve this goal were determining if (1) soil diffusive P concentrations obtained from the soil are affected by residual P fertilizer treatments; (2) soil diffusive P collected has relationships to M3P soil testing; (3) soil diffusive P obtained from the soil is related to tree growth; (4) in vivo sap flow (sap flow P) is affected by residually applied P fertilization; and (5) in vivo translocated sap flow P is related to tree growth or soil diffusive P. To evaluate these objectives, we collected data on diffusive P using the microdialysis technique from two sites previously used as N and P fertilization rate experiments (Tacilla Villanueva 2015) that were harvested in December 2018. The sites were re-established with *P. taeda* in the spring of 2020 to test for residual P fertilization effects. Sap flow P data were collected using microdialysis in *P. taeda* trees, in vivo, under field conditions under specific residual P fertilization treatments during the summer of 2022.

Materials and methods

Site design and treatments

This study is established on two long-term experimental sites, previously used in an N and P fertilization rate experiment established in 1999 by the Forest Productivity Cooperative (formerly Forest Nutrition Cooperative, Albaugh et al. 2015) that was harvested in 2019. Both sites are located on the Atlantic Coastal Plain and represent two different soils (Fig. 2). The first site, referred to as the Alfisol, in Northeast (N.E.) Florida is a poorly drained, refined, mixed, active, thermic, typic Albaqualf (Meggett series) with marine sediment parent material and an argillic horizon. The second site, the Spodosol, is located in Southeast (S.E.) Georgia is a somewhat poorly drained, sandy over loamy, siliceous, active, thermic Typic Haplohumods (Leon series), with marine sediment parent material, with multiple spodic horizons and no argillic or kandic horizon present within the first 100 cm of soil depth. The previous experiment was harvested in 2019 and will be referred to as the "First Rotation." The current rotation, which was overlayed on the same plots as the first rotation, is called the "Second rotation." Experimental treatments at the site level are arranged in a completely randomized block design with two replicates per treatment per site (Table 1 and 2).

Fig. 2 Regional map of each site located in Brantley County, Georgia, and Nassau County, Florida

The First Rotation plots received a range of cumulative fertilizer applications. The First Rotation treatments sampled for this study included four replications of the following treatments: (1) 0 kg P ha^{-1}, (2) 40 kg P ha^{-1}, (3) 60 kg P ha^{-1}, (4) 121 kg P ha^{-1}. The Second Rotation split the four replications of each treatment from the First Rotation into two replications for two different P fertilization treatment groups: 1) "Carryover," no fertilizer P at the establishment for the Second Rotation, 2) "Re-fertilized" with 45 kg P ha^{-1} at the establishment for Second Rotation broadcasted as triple super phosphate treatment notation is the following (X + Y; X = Carryover rate from First Rotation, Y = Re-fertilization rate from Second Rotation; (Table 2). In the Second Rotation, all plots receive N as urea plus a urease inhibitor, potassium (K) as KCl, and a micronutrient mix to remove nutrient limitations besides P. All treatments received 30 ml of Arsenal® herbicide after bedding in the spring for herbaceous weed control.

Soil core and soil microdialysis sampling

Soil samples for diffusive P and Mehlich III (M3P) extractions were collected from two different sites (Spodosol, Alfisol) at two different time points (Year 0 and Year 1) across five treatments (0 + 0 P, 40 + 0 P, 40 + 45 P, 60 + 0 P, and 121 + 0 P) consisting of two replications per treatment, and between

Table 1 Both sites' location, site, and stand properties from the first rotation

Site	County and State	Soil type	Physiographic province	Species	Study establishment	"Base" site index *	Years since P fertilization in 2019	Stand age at harvest (2019)
First rotation 1998 – 2019								
Alfisol	Nassau, Florida (30.6661 N, -81.8361 E)	Meggett	Flatwoods (Pamlico)	*Pinus taeda*	1999	45ft	21	26
Spodosol	Brantley, Georgia (31.3353 N, -81.8217 E)	Seagate	Flatwoods (Penholoway)	*Pinus taeda*	1998	67ft	22	25

The Alfisol study was established at a stand age of three, and the Spodosol was established at a stand age of 5

*Base Site Index is the expected height for that site at 25 years old

Table 2 Treatments and cumulative rates of applied fertilizer for the first and second rotation

All treatments received NPK in the First Rotation. All treatments received N and K in the Second Rotation—only one treatment for sap flow P received fertilization with P

Soil & Sap flow P microdialysis	First rotation carryover rates		Second rotation re-fertilization rates		
	Cumulative P fertilization (kg ha^{-1})	Cumulative N fertilization (kg ha^{-1})	N at planting (kg ha^{-1})	K at planting (kg ha^{-1})	P at planting (kg ha^{-1})
Treatment	Soil Diffusive P				
0 + 0 P	0	0	52	29	0
40 + 0 P	40	400	52	29	0
60 + 0 P	40	600	52	29	0
121 + 0 P	121	1210	52	29	0
Treatment	Translocated Sap flow P				
0 + 0 P	0	0	52	29	0
40 + 0 P	40	400	52	29	0
40 + 45 P	40	600	52	29	45
121 + 0 P	121	1210	52	29	0

Table 3 Sampling points collected for Mehlich 3 (M3P), soil diffusive P, and sap flow P

Sampling	Mature stand (Year 0)	Planting (year 1)	One-year-old heights (year 2)	Two-year-old heights (year 3)
M3P (Yearly)		X	X	
Soil Diffusive P	X	X		
Sap Flow P				X

the bed row and interrow of each treatment plot. The first time point was collected at "Year 0" in the fall of 2018 in a mature stand. The second time point, "Year 1", was collected in March 2020, after planting but before fertilization (Table 3). Two composite samples were collected from each treatment replication: one from the bed (tree row) and one from the interbed (interrow of trees) from each replication. Mineral soil pH (1:1, soil/water by volume) was determined using a combination of glass electrode pH, and cation exchange capacity (CEC) was determined by the Virginia Tech Soil Testing Lab. Each composite soil sample combined eight soil cores to a depth of 0 to 15 cm. Soil samples were dried for two days at 50 °C, sieved using a 2 mm mesh, and stored at -20 °C for future analysis. Soil samples collected were tested for acid-extractable P using the M3P method and analyzed via ICP-MS (Waters Agriculture Lab. Camilla, GA). Note: The 40 + 45 treatment is excluded from analysis in Year 0 and Year 1 because it has not yet received additional fertilization at the time of sampling.

Tree height sampling

Each treatment replicate contains six rows with 12 trees per row for ~ 70 trees per treatment. All 70 trees in the treatment plots were tagged with aluminum tags to track individual tree growth and mortality over time. For the Second Rotation (2019-present), individual tree heights, root collar diameter, and mortality were collected for each measurement plot in the first two years of growth for the Alfisol and the Spodosol. Trees were measured by hand and collected during the winter of January 2021 and January 2022, approximately one and two years after establishment. At this early stage in growth, tree height and root collar diameter were highly correlated; therefore, tree height was used as our indicator for P responsiveness.

Soil microdialysis sampling

The microdialysis system used for soil diffusive P under laboratory conditions consisted of a single CMA 4004 Syringe Pump with four 5 mL micro syringes loaded with perfusate solution composed of 15 mmol L^{-1} of potassium nitrate (KNO_3) as a counter anion and one mmol L^{-1} of citrate, pH was held at 5.8 ± 0.3 using sodium citrate to buffer. Concentrations of KNO_3 and citrate used in the perfusate were modeled after Demand et al. (2017), which had roughly ~ 25% relative recovery rates of PO_4^{3-} when pumped at 2 µL per minute in stirred solutions. Paired microdialysis probes 10 mm long, 500 µm outer and 400 µm inner diameter with a 20 kDa molecular weight cut-off permeability of the membrane (CMA 20; CMA Microdialysis, Solna, Sweden) were inserted and taped down into empty 50 ml beakers. The soil was then packed around the probes so as to not damage the sensitive microdialysis membranes to field values of bulk density (Table 4, Fig. 3). Deionized water was added until the soil was fully saturated. A total of 2.5 mL of perfusate solution was pumped through each soil probe at a consistent rate of 2 µL per minute for a final dialysate volume of 5 mL, which lasted 20 h and 41 min. Samples containing less than 4 ml of dialysate were re-analyzed, and the associated probes were re-assessed and cleared using a 50% ethanol/DI water solution followed by a full 2.5 ml flush of perfusate with no soil medium. Probes yielding less than 2 ml by the end of the flush were discarded as broken. All probes were flushed with perfusate solution for 2 h before and after each sample was collected. The recovery of P collected from these samples was used as our concentration measurements because we were primarily interested in whether variable rates of phosphorus in the soil influenced the concentration of P in the probes. To reiterate, this is not a diffusion-based modeling experiment. We are primarily interested in standardizing a method that can be tested across a wide range of soils to assess concentrations of PO_4^{3-} in the soil in real time.

Dialysates were collected in two 5 mL falcon tubes with the tops sealed with parafilm to limit evaporation. Dialysates were stored at -20 °C until further downstream analysis. Dialysates were analyzed for PO_4^{3-} on a Seal-Analytical AA3 segmented flow auto-flow analyzer. Diffusive P samples were reported using the recovery concentrations of PO_4^{3-} collected from the dialysate in µg L^{-1}. Sixty-four dialysates were collected from our P fertilization treatment plots, 32 from Year 0, and 32 from Year 1 soil samples collected from the same plots. Nine samples were below the 2.5 µg L^{-1} threshold outside the

Table 4 Mean texture, pH, bulk density, CEC, and P values across treatments for each site and year. Year 1 was collected post-harvest at planting

Site & Year	Texture (sand/silt/clay) (% mass)	Bulk density (g cm^{-3})	pH	CEC (cmol kg^{-1})	P (mg kg^{-1}) (Mehlich 3)
Alfisol					
Year 1	80.39/14.85/6.89	1.5	4.45	4.96	13
Year 2			4.14	6.05	16
Year 3			4.02	7.63	8.46
Spodosol					
Year 1	91.37/6.43/2.19	1.6	4.38	6.41	4.21
Year 2			3.75	7.8	7.8
Year 3			4.57	5.42	5.42

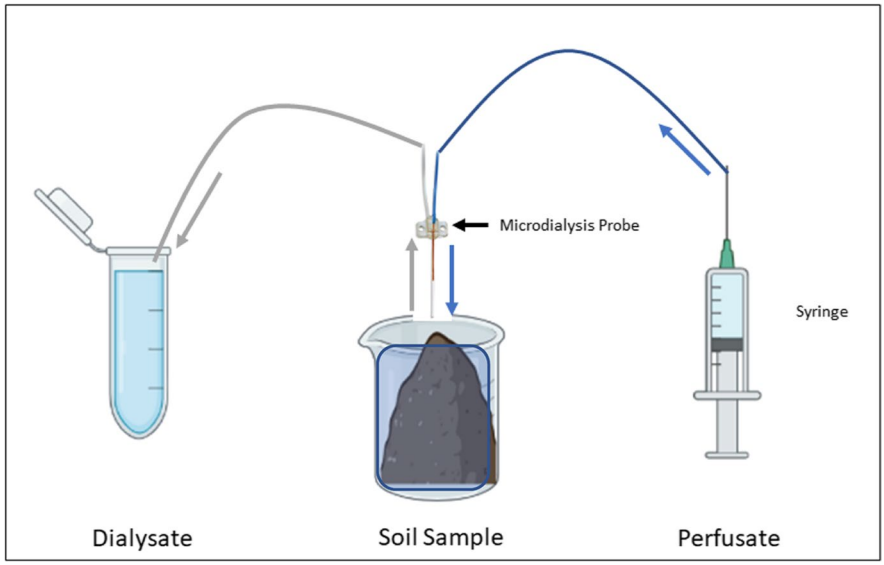

Fig. 3 Simplified representation of microdialysis system to assess diffusive P in soil. The microdialysis probe is fully inserted into a homogenized soil sample slurry of soil and H_2O, and perfusate is pumped into the probe and expelled as dialysate

standard curve and could not be accurately quantified even after repeated attempts. These samples were halved below the 2.5 µg L^{-1} detection limit to 1.25 µg L^{-1} because we could not classify them as missing values. All nine split samples were collected from the Alfisol, not the Spodosol, and seven were collected from Year 0 from the mature *P. taeda* stand (before harvest and replanting).

Sap flow P microdialysis sampling method

The Sap flow P section of this experiment was designed as an exploratory analysis and an initial step in assessing the applicability of deploying microdialysis in-vivo to assess whether (1) The possibility of assessing P concentrations in the sap of *P. taeda* using microdialysis probes and (2) whether those concentrations would be related to treatments and other P fertilization assessment tools. Sap flow P was measured from one tree per treatment replication in the summer of 2022, two years after planting in the following treatments (in kg ha^{-1} P): 0 + 0 P, 40 + 0 P, 40 + 45 P, and 121 + 0 P. Because tree size can influence sap flow, only healthy trees with diameters between 9.5 and 10 cm were chosen. Four microdialysis probes were inserted into the sapwood of sixteen 3-year-old *P. taeda* trees (1 tree per plot, two plots per treatment, four treatments per site, two sites) (Fig. 4). Eight samples were collected from the Alfisol and eight from the Spodosol. Probes were drilled into the base of each tree using a 1 mm drill-bit and flushed using 5 mL of deionized (DI) H_2O. Probes were affixed and sealed to the trees using a silicone paste. Perfusate solution passed through the probes was DI H_2O treated with 15 mmol of KNO_3 to offset the potential osmotic imbalance (Demand et al. 2017). Microdialysis probes were inserted into the base of each probe and sealed around the hole using a silicone sealant. Probes were set to run for four hours at a constant flow rate of 10 µL min^{-1}) until they reached a final volume of approximately 5 mL (Fig. 5).

The same microdialysis pump for diffusive P in the soil was used for sap flow P. Sap flow rates in *P. taeda* can vary drastically on both daily and seasonal scales (Ford et al. 2004). To attempt to account for this, samples collected from each site were collected intermittently over twelve days from June through July 2022. Samples were only attempted on sunny days with minimal cloud cover from 9 a.m. to 1 p.m. and from 1 p.m. to 5 p.m. if the weather stayed constant throughout the day. Weather data was collected daily as probes were monitored over the 4-h run time. Consistent sunny weather was significant because solar radiation influences the sap flow translocation and transpiration rates of the trees (Xia et al. 2008). Eleven of the sixteen samples were set up during sunny periods; however, intermittent or coastal storms caused five samples to be collected during complete cloud cover, which was noted in the field. All

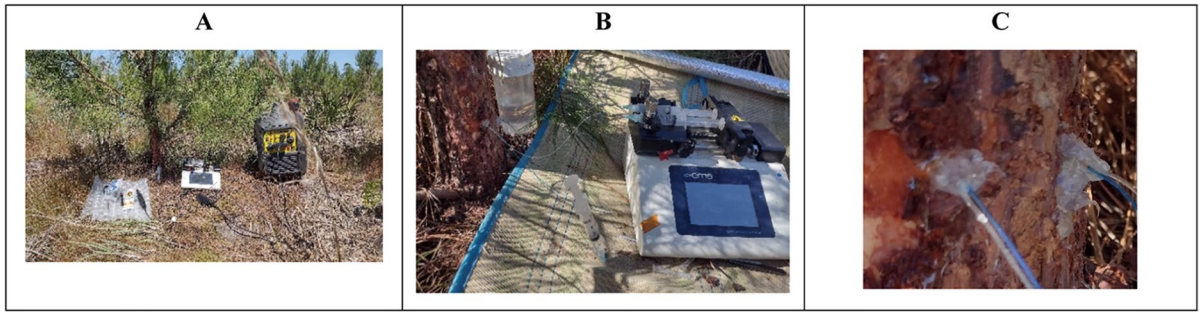

Fig. 4 **A** A Gas-powered generator and microdialysis pump were set up next to each tree after removing the flammable substrate from around the generator. **B** The microdialysis system consisted of four syringes and four microdialysis probes drilled into the tree after carefully removing the outer layer of bark. **C** Each hole was flushed with DI water and sealed using silicone paste to prevent leaking

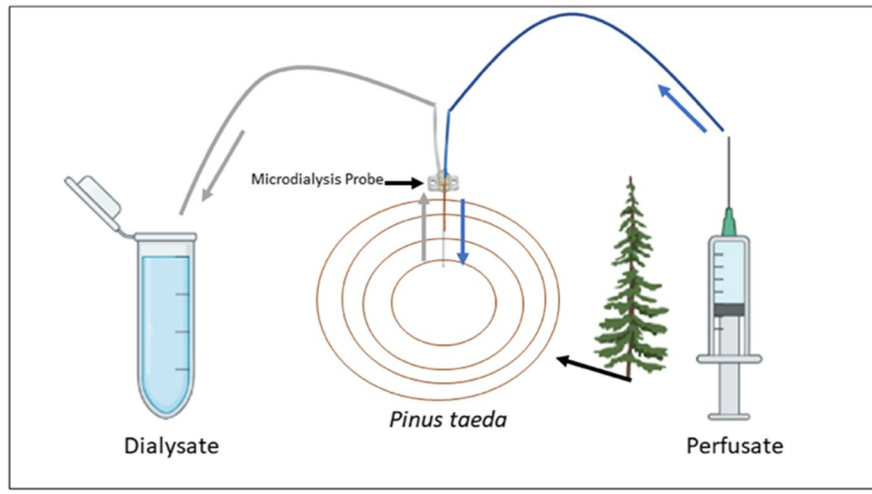

Fig. 5 In vivo microdialysis in a tree. Perfusate solution is passed through a microdialysis probe sealed within the tissue of a P. taeda tree. The resulting dialysate is collected from the sap concentration of PO_4^{3-}. Probes were inserted the entire 20 mm length into the tree, with the top 10 mm holding the semi-permeable membrane

days classified as cloudy were 100% overcast of the total run time. We evaluated tree heights relative to sap flow P concentrations using a standard linear regression to determine if concentrations of P detected in the sap were related to increased tree heights. Unfortunately, only visual assessments were made based on the weather. Photosynthetically active radiation sensors will be deployed to improve the accuracy of further experimentation, but seeing as this was an exploratory experiment to test for the viability of methods, these sensors were not deployed at this stage. These sensors will allow us to correct fluctuations in ambient weather.

Statistics

Diffusive soil P and M3P were analyzed using one-way ANOVAs treating bed and interbed as random effects, and P fertilization treatments were our fixed effect blocked by site and year. Differences between fertilization treatments from Year 0 to Year 1 were analyzed using a two-way ANOVA with P fertilization treatment and treatment year as fixed effects. Carryover treatments were separately analyzed within each treatment year and site using one-way ANOVA. Šidák's multiple comparison tests were applied for each P fertilization treatment and year by site,

considering its conservative nature when comparing unequal sample sizes. Dunnett's multiple comparison tests with an alpha value of 0.10 were used to analyze carryover treatments against the control treatment to assess the impact of P fertilization treatments on diffusive P. For Sap flow P analysis, multiple exploratory one-way ANOVAs were constructed, testing atmospheric conditions, site, and fertilization treatments as fixed effects independently using an alpha value of 0.10. Standard linear regressions using GraphPad Prism v9.0 software were performed to analyze the relationships among M3P, sap flow P, diffusive P, and tree heights collected for two-year-old heights. All other analyses were conducted in JMP Pro v16.0, SAS Institute Inc., Cary, NC, USA.

Results

Carryover and site effects on diffusive phosphorus over time

Mean bulk soil pH values were similar for both sites and years; the Spodosol had a mean pH in the top 15 cm of 4.08 ± 0.06, and for the Alfisol, the pH was 4.48 ± 0.05. The total amount of P recovered from the probes varied greatly by site, P fertilization treatment, and year. In most treatments, recovery concentrations of PO_4^{3-} were over 100 times greater in the Spodosol than in the Alfisol for both Year 0 and Year 1 samples (Fig. 6). Diffusive P samples collected in Year 0, at the end of the First Rotation, averaged across all P fertilization rates, had significantly lower recovery concentrations of PO_4^{3-} than samples taken at Year 1 for both the Spodosol and Alfisol with a 137% increase for the Spodosol and a 166% increase for the Alfisol from Year 0 to Year 1 (p-value < 0.01). Year 0 diffusive P was highly responsive to P treatments for the Spodosol (p-value < 0.01), but diffusive P was unresponsive in the Alfisol at Year 0 (p-value = 0.20). The highest carryover treatment (121 + 0 P) had the lowest overall concentration of PO_4^{3-} at Year 0 samples for the Spodosol. Comparing individual carryover treatments within the site from Year 0 to Year 1 highlights the changes and magnitude of response with increasing rates of P from the First Rotation (Fig. 6).

Year 1 Mehlich 3 results by P fertilization treatment

For soils tested in Year 1, Alfisol had 80–200% increases of P (ppm) detected via M3P compared to the Spodosol for all carryover fertilization treatments regardless of application rate in the top 0–15 cm of soil. The Alfisol 121 + 0 P carryover treatment had a

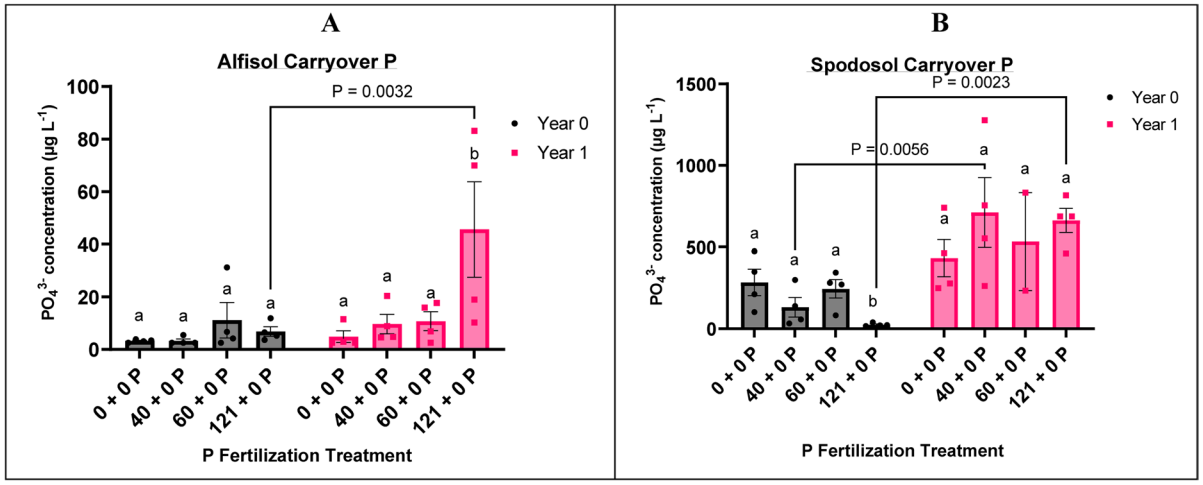

Fig. 6 Diffusive P (PO_4^{3-}) concentrations from Year 0 (black bars) to Year 1 (red bars) varied considerably between sites with significantly less PO_4^{3-} in the Alfisol (**A**) vs. the Spodosol (**B**). Responses from Year 0 to Year 1 varied between carryover treatments. Šidák's multiple comparisons tests showed significant responses within carryover treatment from Year 0 to Year 1. Within site and year, differences showed responses to carryover treatment separated by connecting letters. Error bars represent the standard error of the mean for each treatment within the site and year. Note that Alfisol and Spodosol values are not shown at the same scale

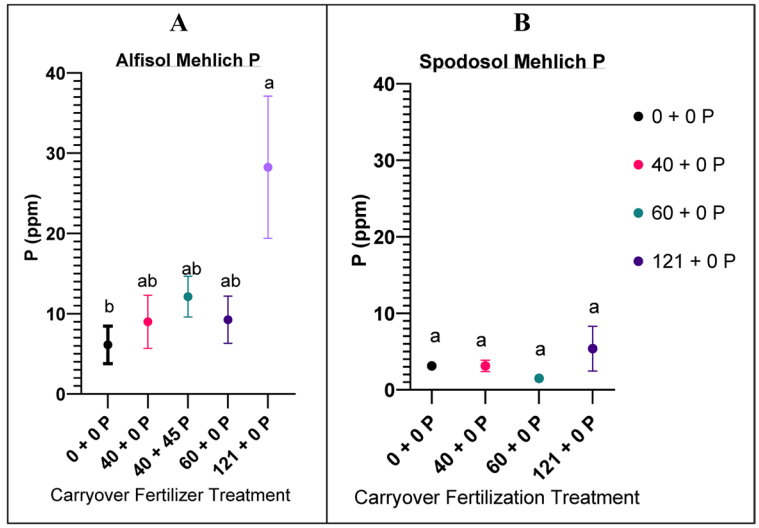

Fig. 7 Year 1 Alfisol and Spodosol Mehlich P values by carryover fertilization treatments. Letters represent Dunnet's multiple comparisons to the control (0 + 0). Error bars represent standard error from the mean at 0.05 alpha value. Note that 40 + 45 treatment has not yet received an additional 45 kg P ha^{-1} fertilization in Year 1 at the time of collection

strong effect on P compared to the control (0 + 0 P). No significant differences between carryover treatments were detected for the Spodosol (Fig. 7).

Soil diffusive P relationships to Mehlich 3 (M3P)

Mehlich 3 and diffusive P collected at Year 1 had a positive relationship in the Alsifol but did not have a relationship in the Spodosol. No significant relationships between relative concentrations of diffusive P were observed for either the Spodosol or the Alfisol for samples collected in Year 0 with M3P samples collected in Year 1 (Fig. 8, Table 5).

Soil diffusive P relationships to tree height

Diffusive P samples collected in Year 1 showed moderate evidence of a relationship to 2-year-old heights for the Alfisol (p-value ≤ 0.01). No association was found between Year 1 diffusive P collected and 1-year-old heights. The Spodosol, although having accumulated significantly more PO_4^{3-} than the Alfisol, showed no evidence of a relationship to either 1 or 2-year-old heights (Fig. 9), possibly indicating the Spodosol was not limited in P.

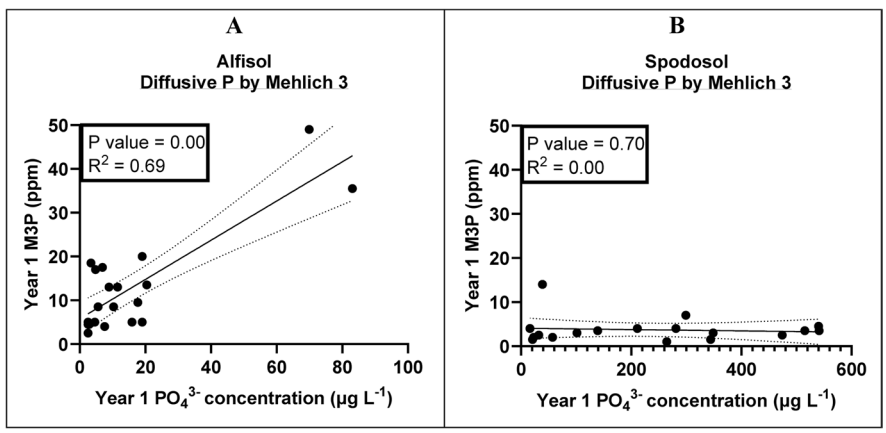

Fig. 8 Year 1 Mehlich 3 (M3P) and diffusive P concentration regression analysis for the Alfisol (**A**) and the Spodosol (**B**)

Table 5 Regression table between concentrations of diffusive P by Mehlich 3 (M3P) and tree heights. Diffusive P samples were collected at Year 0 before harvest of a mature stand, and Year 1 samples were collected post-harvest at planting of the second rotation. Mehlich 3 samples used in this analysis were also collected in Year 1

(*) Significant 0.05 alpha

Year and Site	R^2	F	DFn, DFd	*P*-value	Equation
Mehlich 3 by Diffusive P					
Year 0 Alfisol	0.02	0.40	1, 18	0.54	Y = −0.08077*X + 7.021
Year 1 Alfisol	0.69	41.90	1, 18	0.00*	Y = 1.563*X − 4.286
Year 0 Spodosol	0.05	0.16	1, 16	0.35	Y = -6.483*X + 260.2
Year 1 Spodosol	0.00	0.00	1, 16	0.70	Y = 1.146*X + 536.8
Tree Heights by Diffusive P					
Year 0 Alfisol	0.06	1.04	1, 17	0.32	Y = 0.007301*X + 1.983
Year 1 Alfisol	0.29	7.02	1, 17	0.02*	Y = 0.004819*X + 1.951
Year 0 Spodosol	0.04	0.68	1, 16	0.42	Y = 0.0002140*X + 1.904
Year 1 Spodosol	0.00	0.01	1, 16	0.94	Y = 1.291e-005*X + 1.948

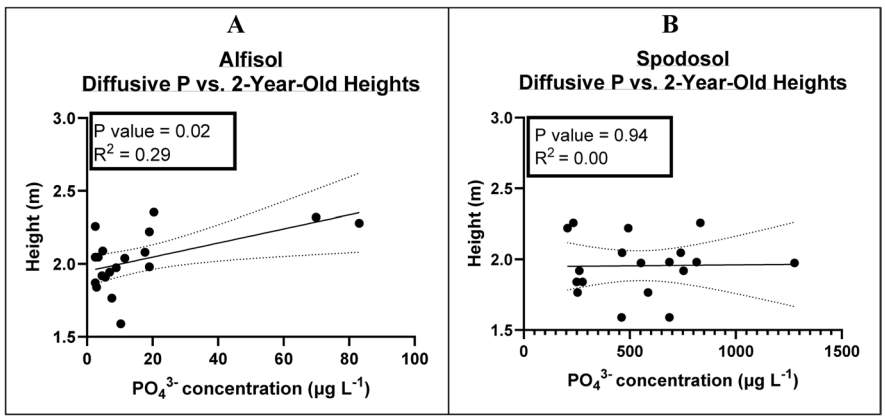

Fig. 9 The Alfisol (**A**) height was positively related to diffusive PO_4^{3-} recovery concentrations for two-year-old heights. Spodosol (**B**) height was not associated with diffusive PO_4^{3-} concentrations

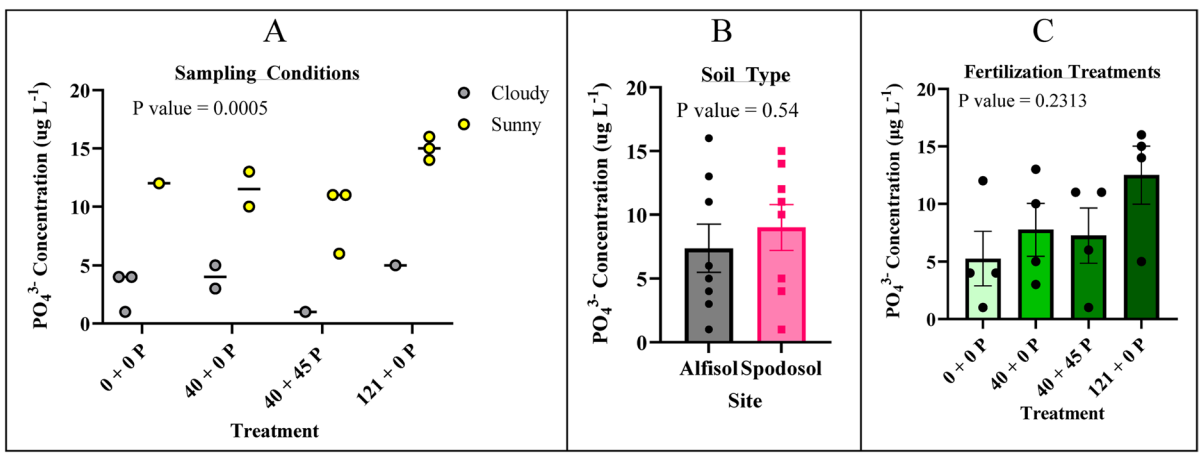

Fig. 10 **A** In vivo sap flow P recovery concentrations results show samples collected during sunny weather (yellow circles) accumulated significantly more PO_4^{3-} in the sap than during cloudy days (gray circles) across carryover treatments (0 + 0 P, 40 + 0 P, 121 + 0 P) and re-fertilized (40 + 45 P). No significant differences were found for any individual treatments. Bars represent the mean for each treatment. **B** Sap flow P relative recovery concentrations by soil type. **C** Sap flow P recovery concentrations by fertilization treatments. Each point represents a single observation; error bars represent the standard error of the mean

Detecting translocated sap flow P using microdialysis probes

Initially, a robust relationship was found between PO_4^{3-} recovery concentration and cloud cover, with significantly higher PO_4^{3-} recovery concentrations on sunny days vs. cloudy days (p-value = 0.0005) (Fig. 10a). Night and day cycles and solar radiation levels throughout the day are known to influence sap flow by up to 100% (Xia et al. 2008; Jian et al. 2019); because of this, we also suspect that the reduction in sap flow led to a proportional decrease in PO_4^{3-} recovery concentrations in the probes on cloudy days vs. sunny days. No significant shifts were observed in sap flow P for either the Alfisol or the Spodosol, and no significant relationships to increases in P fertilization treatments were detected either (Fig. 10bc).

Translocated sap flow P relationship to tree heights

Sap flow P concentrations had a moderate relationship to 2-year-old heights for the Alfisol but not the Spodosol. We did not find any relationships between the concentration of sap flow P and the recovery concentration of soil diffusive P collected in either Year 0 or Year 1 (Fig. 11).

Sap flow P and Mehlich P

Mehlich soil data was collected from Year 1 and Year 2 from the Alfiosl and the Spodosol. Year 1 mehlich samples were positively correlated with Sap flow P, but not from samples collected from Year 2. Samples grouped by soil type did not have a significant relationship to Sap flow P using a 0.05 alpha value but did show a weak relationship of 0.07 (Table 6).

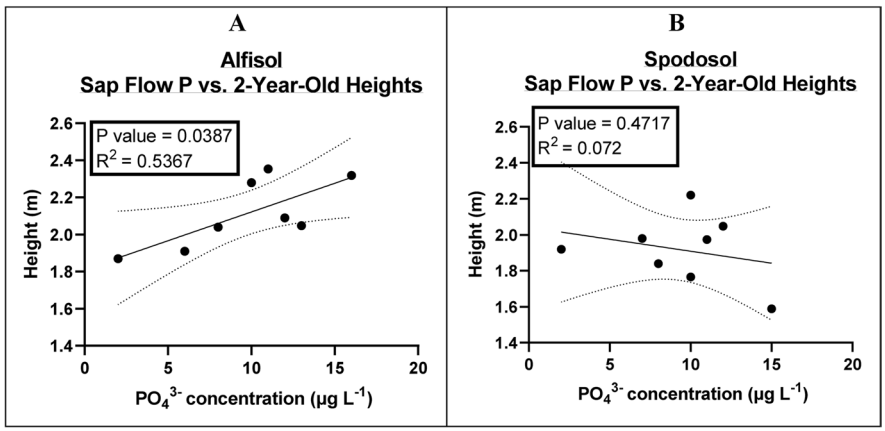

Fig. 11 Sap flow P predicted tree heights with solid confidence in the Alfisol (**A**) but had poor Spodosol (**B**) prediction

Table 6 Sap flow P relationships to Mehlich P collected from Year 1 and Year 2. Sap Flow P was collected in Year 3, one year after Mehlich data was collected

Site and Year	R^2	F	DFn, DFd	P-value	Equation
By Year					
Year 1 Mehlich	0.40	7.98	1, 12	0.02*	Y = 0.3239*X + 7.503
Year 2 Mehlich	0.06	0.75	1, 12	0.40	Y = -0.1139*X + 11.40
By Site					
Alfisol Year 1 Mehlich	0.45	4.87	1, 6	0.07	Y = 1.435*X + 0.9995
Alfisol Year 2 Mehlich	0.07	0.47	1, 6	0.52	Y = -0.6232*X + 19.11
Spodosol Year 1 Mehlich	0.44	4.76	1, 6	0.07	Y = 0.3925*X + 0.1949
Spodosol Year 2 Mehlich	0.02	0.10	1, 6	0.76	Y = 0.04597*X + 8.044

(*) Significant at 0.05 alpha value

Discussion

To our knowledge, this study is the first to deploy microdialysis to measure and assess its applicability for detecting and quantifying soil diffusive P in *P. taeda* silviculture systems. The objective of using this method was to assess whether concentrations of solution and diffusive phase P, detected via microdialysis, could be used to make fertilization land management decisions in silvicultural systems. To reach this objective, we used previously optimized perfusate solutions and flow rates based on Demand et al. (2017) to increase the recovery rates of P across two soil types. These recovered rates of P from microdialysis were compared against conventional M3P samples collected from the same soils. The success of this experiment was rooted in two hypotheses. H_1: Solution phase P in the soil increases with increasing rates of P fertilization from a previous rotation. H_2: Increases to solution phase P pools result in increases in tree productivity. H_3: Solution phase P concentrations would provide stronger relationships to growth than acid extractable M3P method. Each hypothesis assumed the soils were p-deficient and would be responsive to additional P fertilization and that the perfusate solution and flow rate would represent roughly 20–30% of solution phase P concentrations in each soil. An additional assumption for H_3 was made that the perfusate composition and retro dialysis of citrate would mimic a plant root and provide stronger relationships to plant productivity than the conventional acid extractable M3P method.

Mature stand soil samples collected in the fall of Year 0 contained significantly lower concentrations of diffusive P than samples collected in the same stands one-year post-harvest in Year 1. We attribute this increase in P to the disturbance of harvesting and site preparation, which cycles air and organic material into the subsoil, where it would be quickly mineralized (Everett and Palm-leis 2009). Therefore, in Year 0, we are testing mineral soil P without the incorporation of organic material, which would still be unincorporated on the surface, and Year 1 would have both the mineral soil and organic material incorporated into the soil samples. We expected that the previously applied P rates would influence the magnitude of diffusive P seen from this incorporation and mineralization of organic material on the surface. We found this increase in magnitude from Year 0 to Year 1 for both the Alfisol and the Spodosol, indicating that microdialysis was detecting the increase in diffusive P from the incorporation and aeration of this organic material from these disturbance effects (Fig. 6).

From these data, using diffusive P concentrations to detect the magnitude of response from previously applied P rates is highly soil- and fertilization-rate dependent. The 121 + 0 fertilization treatment in the Alfisol contained higher diffusive P than any of the other P fertilization treatments in Year 1, while the 121 + 0 fertilization treatment was the lowest in the Spodosol in Year 0. Concentrations of diffusive P in the Spodosol also suggest the incorporation of organic material from Year 0 to Year 1 was influenced by P fertilization rate increases when compared to the control, referenced by significant increases in the 40 + 0 and 121 + 0 treatments when compared to the control. The effect of P fertilization rates on concentration from Year 0 to Year 1 was also observed in the Alfisol, but only for the highest 121 + 0 treatment. Indicating that the Alfisol requires significantly more applied P in the system before increases in solution or diffusive P are observed.

For our second objective, M3P results from the top 15 cm of soil collected in Year 1 for the Alfisol showed sufficient P above the commonly used five ppm critical level for fertilization (Fig. 7) but not for the Spodosl in Year 1 (Wells et al. 1986). The results revealed relatively similar amounts of M3P from the Alfisol and the Spodosol in the first year of growth, except for the carryover 121 + 0 P treatments on the Alfisol, which was significantly higher than the control treatments. A strong positive relationship was observed between M3P and diffusive P for the Alfisol but not the Spodosol. However, soil diffusive P concentrations were 100 times higher in the Spodosol than the Alfisol across all time points and treatments. Our hypothesis for this discrepancy is that the concentrations of citrate and KNO_3 used in the perfusate dramatically affected the mobilization of P in the Spodosol but not the Alfisol (McKay Fletcher et al. 2021). This hypothesis is also supported by Demand et al. (2017), who found that the same concentrations of solutes within the perfusate had variable accumulations of P depending on the sampling environment. Considering the magnitude of P in the Spodosol was not affected by P fertilization treatments for either M3P or diffusive P, we conclude that microdialysis offers little benefit to traditional acid extractable P

testing at this time. Additional research and optimization of microdialysis on Spodosol could precisely refine these results; however, this is beyond the scope of this publication. Potential drivers of these differences between sites are (1) the Spodosol naturally had higher amounts of sorbed inorganic P to soil surfaces that could be liberated thanks to the perfusate composition (Mckay Fletcher et al. 2021); (2) there was higher microbial mineralization of organic matter occurring in the Spodosol than the Alfisol (Achat et al. 2009); or (3) the P in the Alfisol was sequestered into more difficult-to-access forms of P that the perfusate composition could not liberate. These diffusive concentration disparities between sites could also result from combining these three factors. It is uncertain which soil surfaces, or factors determine the rates and amounts of soil diffusive P accumulated by microdialysis probes. Additional research and consideration must be given to soil characteristics and perfusate compositions to optimize this method for P availability testing for forestry and agricultural applications.

Our third objective was to determine if the diffusive P pool had relationships to *P. taeda* tree growth and productivity. As with the previous results, we found relationships highly dependent on sampling timing and site. It appears from these data that the diffusive P pools tested in Year 0 have little to no connection to the growth rates of trees in the subsequent rotations, probably because the litter layer and organic material have not yet had time to be incorporated into the subsurface horizons (Comerford et al. 2002). However, diffusive P data points collected in Year 1 showed moderate evidence of a positive relationship between increases in diffusive P and height responses for the Alfisol two years after planting. Previous studies demonstrated that microdialysis is a powerful tool for evaluating nitrogen pools in Norway Spruce (Shaw et al. 2014; Inselsbacher and Näsholm 2012), P availability in soil solutions (Demand et al. 2017), and its spatial and temporal similarities to plant roots (McKay Fletcher et al. 2021). These studies have demonstrated that microdialysis could have far-reaching implications in studying mass flow and diffusive nutrients in agriculture, forestry, and environmental samples. Microdialysis probes provide researchers with a pseudo-rhizosphere to simultaneously efflux-controlled amounts of solutes outward from the probe (in our case, citrate) and to passively collect solutes by generating an infinite diffusive sink for specific nutrients in the soil (McKay Fletcher et al. 2021).

To our knowledge, this is the first study to evaluate the use of in situ microdialysis probes to study sap flow P concentrations in *P. taeda* and the second in any tree species. Attempts were made to specifically target only days with high forecasted solar radiation, intermittent clouds, and rainfall throughout the day during multiple sampling events, influencing the total PO_4^{3-} accumulated by the probes (Xia et al. 2008; Jian et al. 2019). The diurnal changes to sap flow in *P. taeda* have been verified and correlate well with temperature, solar radiation, and vapor pressure deficit (Ford et al. 2004). Although these data were not directly recorded in this experiment, a general assessment of cloud cover was our best metric to correct for differences in P recovery within the sap. Using this cloud cover assessment data, we assume that to explain the significant decreases in sap flow P concentrations on those days specifically. Though our sample size was limited, the absence of any relationship between diffusive P and sap flow P for either site implies that the diffusive P pools from the soil do not represent the total P distributed in the sap.

Additionally, it is currently unclear as to whether this P obtained from the sap originated from the heartwood or sapwood, as the holes drilled into the tree would have penetrated each layer. Alfisol heights collected at Year 2 show a positive relationship to sap flow P but not for the Spodosol. Based on our diffusive P data, we hypothesize that the Spodosols were not as P-limited as the Alfisols, and thus, we see a more robust response to increasing rates of P in the Alfisol but not the Spodosol. This relationship shows that there could be a robust temporal component that we currently do not have enough data to uncover. Future height measurements and soil sampling with these and additional sites will reveal whether some data points are artifacts or actual responses that need further exploration.

Limitations

Soil microdialysis

A total of 18 probes were broken for the diffusive P soil experiments over the testing of 80 soil samples. Because we did not take the relative recoveries of the

dialysate volumes after microdialysis samples were completed, only a visual assessment, we were limited in making any assumption as to the relative recovery rate of P from each soil. For the scope of our experiment, we focused on the effects of treatments on the concentrations of microdialysis P by soil type; however, comparing across soil types should be treated with caution as the recovery concentrations of P varied between the Spodosol and the Alfisol. Demand et al. (2017) provides a roughly 25% relative recovery of P from their perfusate solution but only on stirred solutions, not in water-saturated soil conditions. Those seeking to replicate the methodology used in this experiment should be aware that measuring dialysate recovery volumes before discarding samples is imperative to calibrate probes and determine relative recovery of P concentrations. Additionally, pooling probes on each sample made calculating the dialysate relative recoveries of individual probes impossible. Future experiments should contain one probe per sample for individual calibration for dialysate recoveries. Fully saturating the soil also could have dramatically influenced the efflux of citrate into the surrounding medium by removing the water potential difference between the probe and the surrounding medium. Because we did not test for the recoveries of citrate or P in the dialysates, it is impossible to assert that citrate had a viable effect on the relative recoveries of P from either soil. To ameliorate these problems, a control solution with no citrate or KNO_3 have been advised to test for differences between solutions. To reiterate, the composition of the dialysate was chosen solely based on the most optimal conditions found by Demand et al. (2017) to optimize the recovery of P from the soil solution across multiple soil types, not to mimic or model root-soil-probe dynamics.

Sap flow

A total of 12 probes were used throughout the sap flow P microdialysis experiments. Almost all probes were broken by the end of the investigation due to the fragile construction of the probes themselves. We initially expected to lose some of the probes in the extraction process from the trees, and because each probe can return variable relative recoveries of P in solution (Demand et al. 2017), we used four probes for each sampling pool and had strict adherence to the time-intervals within each tree. Extraction from the trees after each run had the highest probability of probe failure. Other shortfalls of the sap flow experiment included fluctuations in weather, which affected the sap flow P rates of the trees. To improve upon these results, the implementation of a photosynthetically active radiation sensor will dramatically improve our corrections to atmospheric conditions. The final limitation of this experiment is that there is no current data on the relative recovery rates of P using our perfusate composition in *P. taeda,* as this was meant to be an exploratory analysis for potential future assessments of such work.

Conclusion

Further optimization areas of study will be important in identifying perfusate compositions and flow rates that provide optimal recovery of targeted compounds in environmental settings. Deciding which depths and soil horizons to analyze with the probes could also be crucial when assessing soil deficiencies. These results highlight the applicability of these probes to assess diffusive P supply in the soil and in the sap of trees *in-vivo*. Our data suggests that both the Spodosol and the Alfisol are responsive to the applied magnitude of carryover P fertilization rates from the previous rotation, however, it also appears that neither site is currently P limited by the 2nd year of growth. It's promising to note the Alfisol M3P and 2-year-old heights of trees were correlated with diffusive P collected at planting indicating timing of sampling may play a key role in fertility management of forest ecosystems. Sap flow P was moderately to weakly related to tree heights, Mehlich P and to carryover fertilization treatments, our results highlight that microdialysis probes could have broader applications in the study of nutrient movement and allocation for tree physiology related applications. Additional work on how these diffusive P rates relate to overall plant growth later in the stand's rotation could help elucidate long-term relationships between available P and stand growth.

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Author contributions All authors contributed to the study conception and design. All authors provided editing and suggestions after the first draft. Material preparation, data collection and analysis were performed by Jacob Hackman. The first draft of the manuscript was written by Jacob Hackman and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available in the Springer Nature data repository: Links will be available upon review of data by Springer team.

Declarations

Competing interests There are no competing interests that the author or co-author are knowingly aware of.

References

Achat DL, Bakker MR, Morel C (2009) Process-based assessment of P availability in a low P sorbing forest soil using isotopic dilution methods. Soil Sci Soc Am J 73(6):2131–2142

Albaugh TJ, Alvarez J, Rubilar RA, Fox TR, Allen HL, Stape JL, Mardones O (2015) Long-term *Pinus radiata* productivity gains from tillage, vegetation control, and fertilization. For Sci 61(4):800–808

Barrow NJ (1978) The description of phosphate adsorption curves. J Soil Sci 29(4):447–462

Bhat K, Nye PH (1973) Diffusion of phosphate to plant roots in soil: I. quantitative autoradiography of the depletion zone. Plant Soil 38:161–175

Clark CJ, Holland PT, Smith GS (1986) Chemical composition of bleeding xylem sap from kiwifruit vines. Ann Bot 58(3):353–362

Comerford NB, De Barros NF (2005) Phosphorus nutrition of forest trees. Phosphorus: Agriculture and the Environment 46:541–558

Comerford NB, McLeod M, Skinner M (2002) P form and bioavailability in the pine rotation following fertilization: P fertilization influences P form and potential bioavailability to pine in the subsequent rotation. For Ecol Manage 169(3):203–211

Demand D, Schack-Kirchner H, Lang F (2017) Assessment of diffusive phosphate supply in soils by microdialysis. J Plant Nutr Soil Sci 180(2):220–230

Everett CJ, Palm-Leis H (2009) Availability of residual P fertilizer for loblolly pine. For Ecol Manage 258(10):2207–2213

Ford CR, Goranson CE, Mitchell RJ, Will RE, Teskey RO (2004) Diurnal and seasonal variability in the radial distribution of sap flow: predicting total stem flow in Pinus taeda trees. Tree Physiol 24(9):951–960

Fox TR, Miller BW, Rubilar R, Stape JL, Albaugh TJ (2011) Phosphorus nutrition of forest plantations: the role of inorganic and organic phosphorus. Phosphorus in action: Biological processes in soil phosphorus cycling, pp 317–338

Gent JA, Allen HL, Campbell RG, Wells CG (1986) Magnitude, duration, and economic analysis of loblolly pine growth response following bedding and P fertilization. South J Appl for 10(3):124–128

George E, Marschner H, Jakobsen I (1995) Role of arbuscular mycorrhizal fungi in uptake of P and nitrogen from soil. Crit Rev Biotechnol 15(3–4):257–270

Grant C, Bittman S, Montreal M, Plenchette C, Morel C (2005) Soil and fertilizer P: Effects on plant P supply and mycorrhizal development. Can J Plant Sci 85(1):3–14

Hedley MJ, Stewart J, Chauhan B (1982) Changes in inorganic and organic soil P fractions induced by cultivation practices and by laboratory incubations. Soil Sci Soc Am J 46(5):970–976

Holford I (1997) Soil P: Its measurement, and its uptake by plants. Soil Research 35(2):227–240

Inselsbacher E, Näsholm T (2012) A novel method to measure the effect of temperature on diffusion of plant-available nitrogen in soil. Plant Soil 354:251–257

Jeřábek J, Rinderer M, Gessler A, Weiler M (2020) Xylem sap P sampling using microdialysis—a non-destructive high sampling frequency method tested under laboratory and field conditions. Tree Physiol 40(11):1623–1638

Jian S, Wu Z, Hu C (2019) Estimation of water use of *Pinus tabulaeformis carr.* in loess plateau of northwest China. J Hydrol Hydromech 67:271–279

Jungk A (2001) Root hairs and the acquisition of plant nutrients from soil. J Plant Nutr Soil Sci 164(2):121–129

Kalant H (1958) A microdialysis procedure for extraction and isolation of corticosteroids from peripheral blood plasma. Biochemical Journal 69(1):99

Lima JD, Da Matta FM, Mosquim PR (2000) Growth attributes, xylem sap composition, and photosynthesis in common bean as affected by nitrogen and phosphorus deficiency. J Plant Nutr 23(7):937–947

Mason S, Hamon R, Zhang H, Anderson J (2008) Investigating chemical constraints to the measurement of P in soils using diffusive gradients in thin films (DGT) and resin methods. Talanta 74(4):779–787

McKay Fletcher DM, Shaw R, Sánchez-Rodríguez AR, Daly KR, Van Veelen A, Jones DL, Roose T (2021) Quantifying citrate-enhanced phosphate root uptake using microdialysis. Plant Soil 461:69–89

Mehlich A (1984) Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun Soil Sci Plant Anal 15(12):1409–1416

Menezes-Blackburn D, Giles C, Darch T, George TS, Blackwell M, Stutter M, Haygarth PM (2018) Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. Plant Soil 427:5–16

Mosetlha K, Torto N, Wibetoe G (2007) Determination of Cu and Ni in plants by microdialysis sampling: Comparison of dialyzable metal fractions with total metal content. Talanta 71(2):766–770

Oburger E, Leitner D, Jones DL, Zygalakis KC, Schnepf A, Roose T (2011) Adsorption and desorption dynamics of citric acid anions in soil. Eur J Soil Sci 62(5):733–742

Petroselli C, Williams KA, Ghosh A, McKay Fletcher D, Ruiz SA, Gerheim Souza Dias T, ... Roose T (2021) Space and time-resolved monitoring of phosphorus release from a fertilizer pellet and its mobility in soil using microdialysis and X-ray computed tomography. Soil Sci Soc Am J 85(1):172–183

Plassard C, Becquer A, Garcia K (2019) Phosphorus transport in mycorrhiza: how far are we? Trends Plant Sci 24(9):794–801

Plock N, Kloft C (2005) Microdialysis—theoretical background and recent implementation in applied life-sciences. Eur J Pharm Sci 25(1):1–24

Polglase PJ, Attiwill PM, Adams MA (1992) Nitrogen and phosphorus cycling in relation to stand age of *Eucalyptus regnans F. Muell: III.* Labile inorganic and organic P, phosphatase activity and P availability. Plant Soil 142:177–185

Pretti L, Bazzu G, Serra PA, Nieddu G (2014) A novel method for the determination of ascorbic acid and antioxidant capacity in Opuntia ficus indica using in vivo microdialysis. Food Chem 147:131–137

Pritchett WL, Comerford NB (1982) Long-term response to P fertilization on selected southeastern coastal plain soils. Soil Sci Soc Am J 46(3):640–644

Pritchett WL, Swinford KR (1961) Response of slash pine to colloidal phosphate fertilization. Soil Sci Soc Am J 25(5):397–400

Raghothama KG, Karthikeyan AS (2005) Phosphate acquisition. Plant Soil 274:37–49

Ryan PR, James RA, Weligama C, Delhaize E, Rattey A, Lewis DC, Wang E (2014) Can citrate efflux from roots improve P uptake by plants? testing the hypothesis with near-isogenic lines of wheat. Physiol Plant 151(3):230–242

Santner J, Smolders E, Wenzel WW, Degryse F (2012) First observation of diffusion-limited plant root P uptake from nutrient solution. Plant, Cell Environ 35(9):1558–1566

Schachtman DP, Reid RJ, Ayling SM (1998) P uptake by plants: From soil to cell. Plant Physiol 116(2):447–453

Shaw R, Williams AP, Jones DL (2014) Assessing soil nitrogen availability using microdialysis-derived diffusive flux measurements. Soil Sci Soc Am J 78(5):1797–1803

Sibbesen E (1978) An investigation of the anion-exchange resin method for soil phosphate extraction. Plant Soil 50:305–321

Siebrecht S, Herdel K, Schurr U, Tischner R (2003) Nutrient translocation in the xylem of poplar—diurnal variations and spatial distribution along the shoot axis. Planta 217:783–793

Stevenson FJ, Cole MA (1999) Cycles of soils: carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley & Sons

Sung J, Sonn Y, Lee Y, Kang S, Ha S, Krishnan HB, Oh T (2015) Compositional changes of selected amino acids, organic acids, and soluble sugars in the xylem sap of N, P, or K-deficient tomato plants. J Plant Nutr Soil Sci 178(5):792–797

Tacilla Villanueva A (2015) Impacts of Fertilization on Soil Properties in Loblolly Pine Plantations in the Southeastern United States (Doctoral dissertation, Virginia Tech)

Wells CG, Craig JR, Kane MB, Allen HL (1986) Foliar and soil tests for the prediction of P response in loblolly pine. Soil Sci Soc Am J 50(5):1330–1335

Xia G, Kang S, Li F, Zhang J, Zhou Q (2008) Diurnal and seasonal variations of sap flow of *Caragana korshinskii* in the arid desert region of North-West China. Hydrol Process 22(8):1197–1205

Zhang TQ, Zheng ZM, Drury CF, Hu QC, Tan CS (2020) Legacy P after 45 years with consistent cropping systems and fertilization compared to native soils. Front Earth Sci 8:183

Zhu J, Li M, Whelan M (2018) P activators contribute to legacy P availability in agricultural soils: A review. Sci Total Environ 612:522–537