Is it possible to cut down the nutrients release in a Mediterranean drained peatland using C4-turfgrasses species?

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Abstract

Dry matter production, nutrient uptake and tissue nutrient concentration of two C4-turfgrass species (*Cynodon dactylon* x *Cynodon transvaalensis* (L.) Pers and *Paspalum vaginatum* Swartz) supplied with three different nutrient solutions (C, L, H) in sand and peat culture were compared. The 8 week-experiment was carried out in mesocosms and simulated the conditions of an open field phyto-treatment system located in a Mediterranean drained peatland (Tuscany, IT). The peat was collected in the site and the L solution used, mimicked the drainage water flowing into it.

Three hypotheses were tested: 1) are species efficient in nutrients removal from both solution and substrate? 2) is the peat able to contribute to nutrient loads? 3) is the use of these species suitable in the open-field system?

We found support for these hypotheses. The two species showed a good adaptability to the experimental conditions implying a considerable capability in nutrients removal. *Paspalum* was more efficient in nitrogen uptake mainly in high nutrients availability conditions. On peat we observed a supplementary nutrients uptake by plants. The performances of two C4-turfgrasses extrapolated at field scale seemed to be effective considering the peculiarity of the system.

KEYWORDS: *Cynodon dactylon*, *Paspalum vaginatum*, peat, nitrogen, phosphorus, phyto-treatment, mesocosm.

Introduction

The control of eutrophication phenomena in developed countries has been pursued through a drastic reduction of phosphorus (P) and nitrogen (N) point sources pollution and the implementation of measures aimed at the reduction of nutrients release from diffuse sources, especially agricultural ones (Cooper 1993, Dorioz & Fehri 1994, Wang et al. 2004).

Where the high nutrients inputs to freshwater are related to particular conditions of land such as cultivated organic/peat soils, low water table depth, artificial drainage, etc., the human intervention cannot be limited to mitigation measures but must include concrete actions directed specifically towards the abatement of pollution levels (Van der Molen et al. 1998, Withers &Lord 2002). On the contrary, in the period between the two world wars, the need of new arable lands led to a neglectful use and management of peatlands and palustrine areas nearby. The drainage of marshes by the creation of an extensive network of drainage canals and pumping stations (land reclamation)(Pistocchi et al. 2012), have impaired the natural functions of these ecosystems which functioned before as sinks, buffers and filters for nutrients and waters.

The drainage of peats are followed by significant changes in their physical and chemical properties (Litaor et al. 2008). High nitrate (NO_3^-) concentrations in the pore water of drained peatlands are caused by aeration of the peat and subsequent mineralization and nitrification of organic nitrogen (Tiemeyer et al. 2007). In the case of P, aeration causes the mineralization of organic P compounds, which are then frequently sorbed to Fe(III)-hydroxides and thus become temporarily immobilized (Zak et al. 2004). The main consequences are an accelerated organic matter oxidation (with consequent increase of CO_2 emission to the atmosphere) (Blodau 2002), an enhancement of subsidence rate and higher nutrients losses to water bodies (Foley et al. 2005, Schipper &McLeod 2002, Tiemeyer et al. 2007, Verhoeven &Setter 2010).

There are several options to restore drained paetlands: re-wetting with or without topsoil removal (Klimkowska et al. 2010a, Klimkowska et al. 2010b, Zak et al.), constructed wetlands (Brix 1997, Hu et al. 2010), vegetation filters (Pistocchi et al. 2009), paludiculture, i.e. the wet cultivation of marshland, (Wichtmann &Couwenberg 2013). The majority of these strategies involve the use of plants (Silvan et al. 2004). Plants, indeed, allow the nutrient removal through biomass accumulation, fixation of inorganic and organic particulates and the increase of microbial activity in the soil, for example creating an oxidized environment close to the rhizosphere when the growth substrate is saturated.

Therefore it is important to know the adaptability of the plants to saturated soil conditions and their potential nutrients uptake in order to possibly use them on a large scale. The study of plants' performances is, however, very complex if carried out in open field conditions because of the many factors of variability interacting with each other (soil heterogeneity, weeds competition, weather variability, water table fluctiations, etc.) and the difficulty to directly measure some variables (water consumption, contribution of the roots, etc.).

For these reasons, many authors decided to evaluate the efficiency of individual species in uptaking nutrients losses at the laboratory scale, using simplified models of reality, called microcosms or mesocosms (depending on the size), where the interpretation of results becomes more reliable (Fraser et al. 2004). For example Huett et al. 2005 used polyethylene tubs (0.6 m long x 0.37 m wide) filled with 10 mm basaltic gravel to simulate a subsurface flow reed bed treating plant nursery runoff and compared vegetated *vs* unvegetated treatment. They found percentage of removal higher than 90% for both total P and total N in vegetated conditions, while the same percentage where lower than 16% and 45% for total P and N respectively in unvegetated conditions. Similarly Fraser et al. 2004, Polomski et al. 2007 and Polomski et al. 2009 tested different wetland and garden aquatic species in microcosms in order to mimic subsurface flow constructed wetland under different nutrient supply.

Some authors showed that also turfgrass species, mostly C4 species, are efficient in nutrient removal from wastewaters or waters with high nutrient content (Adeli et al. 2003, da Fonseca et al. 2007, Menzel &Broomhall 2006, Nogueira et al. 2013).

We selected *Cynodon* e *Paspalum* for their ease of propagation, remediating ability (Duncan &Carrow 2000), efficiency in nutrient adsorption (Cole et al. 1997, Soldat &Petrovic 2008) and their adaptability to the transition zone of the Mediterranean region (Volterrani et al. 2008).

These two species have similar morphological and ecological characteristics. *Cynodon* has a well developed root system and therefore may survive for long periods of drought. It grows on any soil, it tolerate a wide range of pH condition (5.5-8.5) (McCarty 2002) and it doesn't show any significant reduction of growth at salinity level of 10 dS/m (Beard 2005, Peacock et al. 2004). *Paspalum* is a species characterized by a more rapid settlement than *Cynodon*. It produces a lot of stolons and rhizomes and it presents an exceptional adaptation to salinity conditions (up to 31 dS/m), so it can be considered a halophyte species (Duncan &Carrow 2000). *Paspalum vaginatum* has the ability to withstand long periods of drought but also to tolerate waterlogging (Lee et al. 2005a, Lee et al. 2005b). It survives in a very wide pH range 3.6-10.2 (Duncan et al. 2000).

The aim of this research was to evaluate, at mesocosms scale, the capability of these two C4-turfgrasses species (*Cynodon* and *Paspalum*) to uptake nutrients in agricultural drainage water/released from degraded peat, in relation to their possible use in a large-scale (15 ha) experimental phyto-treatment system. This system is located in a Mediterranean peatland (Vecchiano, Tuscany, Italy), recently rewetted after decades of reclamation and agricultural use in order to treat runoff/drainage water from adjacent cultivated fields, using different rewetting strategies: constructed wetland, restored natural wetland, paludiculture with energy crops and wet meadow (Ciccolini et al. 2013).

Materials and Methods

A greenhouse study was conducted during summer 2013 (from June to August) at Department of Agriculture, Food and Environment, University of Pisa (43° 42' N 10° 24' E).

Experimental Design

The experimental design was a randomized block design (RBD) with three replications.

The main factor was the species with three levels: bare soil (Ba), *Paspalum vaginatum* (Pv), *Cynodon dactylon* x *Cynodon transvaalensis* (Cd), the second factor was the substrate with two levels: sand (Sa) and peat soil (Pe), and the third factor was the nutrition treatment with three levels: C (control solution), L (low input solution) and H (high input solution). The unvegetated mesocosms were only filled with peat soil and treated with L and H solutions, as well as the vegetated mesocosms on sand.

Plant culture and treatment

The turfgrass species selected for the experiment were: *Cynodon dactylon* x *Cynodon transvaalensis* Burtt-Davy 'Tifway' and *Paspalum vaginatum* Swartz 'Salam'.

Three weeks prior to the start of the experiment, uniform plugs of each species (7 cm side, depth: 5 cm) were collected from mature (> 5 years) stands and washed in running tap water to remove the soil.

The mesocosm modeled after (Fraser et al. 2004, Polomski et al. 2007, Polomski et al. 2009) was constituted of a flowerbox (45x20x10 cm), covered with a plastic lid containing three holes, in each of which a pot (surface: 49cm^2, height: 18 cm), with drainage holes on the bottom, was inserted.

Each pot contained in the flowerbox was filled with sand or peat soil (Tab.1) depending on the experimental design, and each plug was transplanted into it (Figure 1). The pots filled with peat soil mimic the field condition of the wet meadow in the above cited experimental phyto-treatment site, since the soil was taken from the field (homogenized and then sieved at 5 mm).

After fitting the three pots per mesocosm, tap water was added to each mesocosm until water reached the level of the overflow hole (6,5 liters). During the acclimation period (three weeks), every 5 days tap water was added to each mesocosm to maintain the water level just below the overflow hole.

At the end of the acclimation period, in the vegetated mesocosms, grasses were mowed at 1.5 cm and the appropriate nutrition treatment was distributed into the mesocosms (vegetated and unvegetated) up to the overflow hole level.

Three nutrition treatments were used: 1) tap water used as control 'C'(P: < LOD; N: 0.73 mg/l), 2) a modified Hoagland solution with a lower level of nutrient 'L'(N: 5.9 and P: 0.10 mg/l), 3) a modified Hoagland solution with a higher level of nutrient 'H'(N: 26.6 mg/l and P: 0.52 mg/l).

The pH, concentrations and ratio of nutrients (P/N = 0.02) in the L solution encompassed the mean values of nutrients found in the water entering the wet meadows in our experimental field. In order to assess the nutrient uptake capability of the species the H solution was 5 times more concentrated than the L solution, maintaining the same P/N ratio. Thereafter, nutrient solution was supplied every 5 days to maintain the level and the volumes added were recorded. Before and after refilling, electrical conductivity (EC) and pH of the solution were measured.

Data collection and statistical analysis

After 8 weeks of treatment, for each mesocosm the above- and below-ground biomasses were separated (after washing with tap water). Dried portions (65 °C to constant dry weight) were weighted and ground separately in a Moulinex Mill. The N and P concentrations of dry biomass were determined in 200 mg of tissue by H_2SO_4/H_2O_2 digestion (Bremner 1965). Nitrogen concentration was determined with Kjeldhal method. Phosphorus concentration was determined by using the blue-molybdenum method, with a Perkin Elmer Lamba 25 spectrophotometer.

On the remaining volume of solution in the mesocosm was performed total phosphorus, N-NO_3 and P-PO_4 analyses.

N and P uptakes were determined by multiplying the above- / below- ground dry weight by relative nutrient concentration. The recovery rate was calculated as the difference between the total nutrients supply (mg of P/N added with nutrient solutions - mg P/N remained in the flowerbox at the end of the experiment) and plant uptakes at mesocosm level.

On the substrates (Sa and Pe) pH, Electrical Conductivity (EC) on the dilute 1:2.5 (soil : water) extract, N Kjeldhal (N-tot), total phosphorus (P-tot) and available phosphorus with Olsen method (P-Ols) were analyzed before and after the experiment. In order to determine accurately P-tot in the peat substrate we tested four different digestion methods. In method A 0.2 g of dry soil were added with 10 ml of H_2SO_4 (97%) and then heated at 400°C for 2h. Method B and method C were similar to method A with a Kjeltab (1.5 g K_2SO_4 and 7.5 mg Se, KJELTABS AUTO 1000/PK TC15270001) or 3 ml of H_2O_2 (35%) respectively added before heating. In method D 0.1 g of dry soil were added with 5 ml of H_2SO_4 (97%) and let settle overnight, then 3 ml of H_2O_2 (35%) were added and the mixture was heated at 400°C for 2h. This last procedure has been tested in order to reduce the amount of sulfuric acid used. Digests were stored at room temperature and analyzed for P-PO_4 as soon as possible.

The different digestion procedures were evaluated taking into account the final aspect of digests and recoveries compared to the ignition digestion method. Samples digested with method A presented a vivid orange color that indicated an incomplete digestion, therefore they were not analyzed fot P-PO_4 concentration. Digests of method C were still lightly yellow, while the ones obtained with method B and D were respectively white and colorless. According to recovery, the digestion methods can be classified as follows: method C < method D < method B. The method B (H_2SO_4 + Kjeltab) allowed to obtain a 100% recovery and has been selected for P-tot analysis.

The evapotranspiration was calculated by performing the water balance of each mesocosm on five days basis (volumes added - volumes left).

A one-way ANOVA was used to test the significance of the treatments on each detected parameter on vegetation and soil, while the mean separation of treatment effects was performed using orthogonal contrasts. All data were processed with R (2.11.1 version, R Foundation for Statistical Computing), and all tests were conducted with $\alpha = 0.05$.

Results

Plant growth and water use

The harvested plant dry biomass was analyzed comparatively between the species, the substrates used and the nutrient treatments (Tables 2, 4 and 5).

The orthogonal contrasts showed that the above-ground biomass (AB) was significantly different between the substrates used (45.5 g and 19.4 g for peat and sand respectively), while the species had no significant effect on this parameter. This trend was also confirmed by the analysis of the others contrasts: Cd and Pv didn't interact significantly with the substrate (peat or sand) and with the nutrient level (L or H solution) inside the peat treatment. Conversely, the interaction between substrate type and nutrient level affected the AB: passing from low to high solution the AB was 4 times higher in the plants rooted in sand and 8 times higher in those rooted in peat.

Unlike the AB, the below-ground biomass (BB) was significantly different between the two species as Cd value was 35% lower than Pv (36.2 g and 55.4 g respectively). The substrate effect was still significant although the two treatments were much closer (43.0 g for peat and 39.1 g for sand) than they were for AB. The only interaction that reached the statistical significance was species *vs* nutrients level in peat: the below-ground biomass of Pv was higher as the nutrients availability was greater (+33.1 g compared to L solution), while the Cd values were substantially equal for both H (45.9 g) and L solution (41.9 g). On sand the same contrast (Cd vs Pv : SaH vs SaL) was statistically negligible. Finally the substrate treatment didn't interact with the species, either with the two nutrient level solutions.

For the crop evapotranspiration (ET_0), we observed the same pattern of the contrasts discussed for the AB, confirming that the water consumption was directly correlated with the above-ground biomass production.

The Water Use Efficiency (WUE) was significantly dependent both on the species and the substrates. Cd was more efficient than Pv (2.2 g/l and 2.0 g/l respectively). Peat determined more favorable conditions for water use by plants than sand (2.4 g/l and 1.7 g/l respectively). No interaction was significant, meaning that the effects of the treatments were additional without showing synergic or antagonistic effects. However the observed values were very heterogeneous ranging from 1.3-2.5 (CdSaL and PvSaL respectively) to 11.0-14.6 g/l (CdPeH and PvPeH respectively).

N and P: tissue concentration, plant uptake and mesocosm recovery

The plant capability to remove nutrients from the mesocosm (the nutrient solutions and/or substrates) was evaluated analysing the nutrients tissue concentrations (NCA, PCA, NCB and PCB), the plants' uptake (NTA, PTA, NTB and PTB) and the recovery rate (nutrients removal balance) on the basis of the tested treatments (Tables 2, 4 and 5 and Figure 2).

NCA was significantly different between species (Cd vs Pv) and for the interaction between species and nutrition level in both substrates as shown by contrasts Cd vs Pv : PeH vs PeL and Cd vs Pv : SaH vs SaL. The N concentration in Pv was always higher than in Cd (11.7 mg/g and 8.7 mg/g respectively). Furthermore, raising the nutrition level (from L to H), the NCA of Pv increased more than Cd: more than 4 times in peat and about two times in sand. No significant effect was due to the type of substrate (10.1 mg/g and 10.4 mg/g for peat and sand respectively).

NCB, conversely, varied significantly depending on the substrate: 6.7 mg/g for peat and 4.7 mg/g for sand. The substrate type also interacted with the species: the increase of the NCB passing from the Pv in peat to Pv in sand is higher than that observed for Cd (+3.2 mg/g in Pv and +0.8 mg/g in Cd). There wasn't any interaction between substrate and solution, either between species and solution in both substrates. The species treatment became significant only for the C solution, in this case the NCB of Pv was higher than Cd (+2.0 mg/g).

The pattern showed by P concentration was quite different. Only the substrate treatment indeed determined significant differences in P content both in above and in below ground biomass (PCA was 1.7 mg/g and 1.0 mg/g in peat and sand respectively and PCB was 1.8 mg/g and 0.8 mg/g in peat and sand respectively). It should be noted that in conditions of limited availability of phosphorus (C solution), the PCA was statistically greater in Pv than in Cd (2.8 mg/g *vs* 1.7 mg/g).

Plant uptake was calculated as described in the Materials and Methods section, considering both the whole biomass (NTT = NTA + NTB = AB x NCA + BB x NCB) and the harvestable biomass (NTA = AB x NCA) produced by mesocosms. The latter represented the real nutrient uptake from the system. The same equations were applied to phosphorus.

NTT was not significantly influenced by the species. Pv uptook 862 mg of N (partitioned in 57% as NTA and 43% as NTB) and Cd uptook 557 mg (partitioned in 64% as NTA and 36% as NTB). Conversely the difference between substrates was significant, with mean value in peat reaching 859 mg (of which 59% in the above-ground biomass) and in sand 423 mg (of which 56% in the above-ground biomass). All the NTT interactions, except Cd *vs* Pv : PeH vs PeL, were significant. The contribution of NTA to those effects was greater than NTB.

Conversely PTT was affected significantly by the species and not by the substrate (109 and 123 mg of P for Pv and Cd respectively) as well as the interaction effects Sa vs Pe : L vs H and Cd vs Pv : PeH vs PeL. However the P uptake was differently partitioned between below and above -ground biomass compared to N uptake, which was higher for the above-ground biomass.

The analysis of recovery rate showed generally a more negative results for the mesocosm with peat, in particular for N (-59 and -15 mg of P for peat and sand respectively; -220 and -30 mg of N for peat and sand respectively). Considering the species, Cd was more efficient in P recovery (+27 mg of P compared to Pv), while Pv showed a greater capability to remove N (+190 mg of N compared to Cd). The effect of peat substrate was confirmed also by the values of balance of PeH (Figure 2). Finally it was evident that, in high availability of nutrients (Pe + H) the two species showed different behavior: high removal of P for Cd (-162 mg) and of N for Pv (-663 mg).

Effect on substrates

In Tables 3, 6 and 7 are listed the results of contrasts concerning soil parameters: available phosphorus (P-Ols), total phosphorus (P-Tot) and total nitrogen (N-tot), pH and EC.

Regarding nutrients the only statistically significant effects were recorded for P-Ols and P-tot between bare and vegetated mesocosms: 25 and 21 ppm of P-Ols for Ba and Veg respectively; 3711 and 2803 of P-tot for Ba and Veg respectively.

Significant differences were observed for the pH of the contrast Ba *vs* Veg and (PeH + PeL) *vs* PeC. These trends could be explained by the absence (Ba) or lower growth (PeC) of plants in the mesocosm.

Discussion

The two species showed a different behaviour considering both biomass production and nutrient removal. *Cynodon dactylon* (Cd) and *Paspalum vaginatum* (Pv) presented moderate to high yields in our experimental conditions (temperature and soil moisture). In particular Pv seemed to perform better in conditions of high nutrients availability (peat substrate treated with H solution). This could be related to a more developed root system of Pv, showing a higher stolons density and total weight.

Considering the N uptake by the above-ground biomass, which represents the effective removal from the system through biomass harvesting, Pv performed better than Cd. This was mainly related to the different nutrients concentrations observed in plant tissues. In the case of NTB instead the better performance of Pv was related mainly to the higher biomass production.

Differences in P concentration between the two species were lower, consequently the differences in P removal were notable only for below-ground biomass.

The comparison of the vegetated treatment with the bare soil, reproducing the simple re-wetting strategy allowed to highlight that vegetated mesocosms performed better than the unvegetated ones with respect to P removal. Indeed both available P and total P content in substrates showed significant reduction in vegetated mesocosms. Other studies using mesocosms came to the same conclusion (Fraser et al. 2004, Huett et al. 2005, Rogers et al. 1991, Tanner 2001, Tanner et al. 1995). It has to be considered that those differences are mainly due to below-ground uptake, consequently this fraction of P cannot be considered as permanently removed from the system.

Finally the highest nutrient removal efficiency has been observed for the combination PvPeH for nitrogen and CdPeH for phosphorus.

The attitude of peat soil to release nutrients was confirmed by the higher values of concentration and uptake observed both in above- and below-ground biomass. Consistently the recovery rate values showed that the amounts of removed nutrients were 4 to 7 times higher in peat under equal nutrients supply.

The effects of solution treatment was tested mainly in relation to the different substrates and within each substrate treatment in relation to the species.

Peat use determined an enhancement of nutrient uptake when combined to a high nutrient supply (H solution). This effect could be explained by the higher availability of nutrients in the supplied solutions, which stimulated plant growth (Polomski et al. 2007, Polomski et al. 2009).

Mesocosms are useful for controlled, mechanistic investigation (Fraser &Keddy 1997) and they have been used to test plant ability to treat wastewater, but they present some limitations in the extrapolation of the results to field scale, as the extrapolation depends greatly on the reference surface chosen, furthermore the experimental conditions may affect their reliability (Fraser et al. 2004).

Nevertheless, we tried to make an extrapolation using data from the combination of treatments reproducing better the field conditions: peat soil and L solution. Considering an area of 0.2 m^2 as reference surface we extrapolate the main measured parameters to a square meter surface: 122 and 120 g/m^2 of AB, 6.9 and 6.5 mm/d of ET_o, 1.1 and 0.9 g/m^2 of NTA, 0.22 and 0.16 g/m^2 of PTA for Pv and Cd respectively. These assumptions allowed to estimate the performances of the two species established in the wet meadow of the above cited experimental field with the following characteristics: the surface available for the turfgrass cultivation is around 20000 m^2, the hydraulic load is about 670 m^3/d and the nutrients loads are equal to 3.96 and 0.067 kg/d of N and P respectively. We estimated an above-ground biomass production of 2.60 and 2.55 t of dry matter for Pv and Cd respectively, a dissipation in atmosphere of 146 and 138 m^3 of water (in the full growing conditions, corresponding to about 20% of the daily water inflow) and a nutrients removal of: 0.42 and 0.34 kg/d of N and 0.08 and 0.06 of P for Pv and Cd respectively. On the basis of these data the estimation of nutrients removal harvest is about 10% for N (10.6 and 8.6 for Pv and Cd respectively) and close to 100% for P. In the case of Pv this rate reaches the value of 124% showing the potential of this species to catch the nutrients released by the soil.

Conclusions

In this paper we presented a mesocosm experiment in which we assessed the capability of two turfgrasses (*Cynodon dactylon* x *Cynodon transvaalensis* (L.) Pers and *Paspalum vaginatum* Swartz) to remove N and P from the soil-plant-water system.

Our results showed that both turfgrasses are efficient in removing N and P, but P. *vaginatum* performed better in removing N. Moreover our results on substrate supported the hypothesis that vegetated conditions are better than simple rewetting (bare soil) in controlling nutrient losses and especially P, as showed by the significantly lower available and total P content in substrate at the end of the experiment.

Even if there are some limitation in extrapolating results to field scale, such as the short growing period of the experiment or possible experimental artifacts, we were able to estimate that the use of turfgrasses allow to reduce (about 10%) the N loads in water and abate almost all P (from 90 to 124%), removing also a part of P released by the soil. It is important to notice that an equivalent or higher (in the case of P) amount of nutrient is immobilized in the root systems, thus temporarily unavailable for leaching.

The use of turfgrasses is quite uncommon in phyto-treatment systems (except for buffer strips) but our experiment showed that they could be successfully used to remove nutrients in saturated conditions by harvesting biomass. Nevertheless the diffusion of turfgrasses at large scale in phyto-treatment systems is constrained by the lack of an established marked chain of these crops. Our experiment also provided useful information for the design of such systems, such as evapotranspiration rate at full growing conditions and expected nutrients removal/immobilization.

Acknowledgements

The authors wish to thank Tiziana Sabbatini (Institute of Life Sciences - Scuola Superiore Sant'Anna) and Monica Gaetani (Department of Agriculture, Food and Environment – Università di Pisa) for their invaluable technical support.

References

Adeli A, Varco J, Rowe D (2003): Swine effluent irrigation rate and timing effects on bermudagrass growth, nitrogen and phosphorus utilization, and residual soil nitrogen. Journal of Environmental Quality 32, 681-686

Beard J (2005): Beard's turfgrass encyclopedia. Michigan State Univ. Press, East Lansing

Blodau C (2002): Carbon cycling in peatlands A review of processes and controls. Environmental Reviews 10, 111-134

Bremner JM (1965): Total Nitrogen. Methods of Soil Analysis. Part 2. Chemical and microbiological properties agronomymonogra, 1149-1178.

Brix H (1997): Do macrophytes play a role in constructed treatment wetlands? Water Science and Technology 35, 11-17

Ciccolini V, Giannini V, Bosco S, Pellegrino E, Pistocchi C, Sabbatini T, Rossetto R, Silvestri N, Bonari E (2013): Restoration of a Mediterranean drained peatland: the case study of the Massaciuccoli Lake Basin (Tuscany, IT). SER Knowledge Database (http://ser.semico.be/ser-pdf/AWARE_extended_abstract_Ciccolini%2020%2011%202013.pdf)

Cole J, Baird J, Basta N, Huhnke R, Storm D, Johnson G, Payton M, Smolen M, Martin D, Cole J (1997): Influence of buffers on pesticide and nutrient runoff from bermudagrass turf. Journal of Environmental Quality 26, 1589-1598

Cooper CM (1993): Biological effects of agriculturally derived surface water pollutants on aquatic systems- a review. Journal of Environmental Quality 22, 402-408

da Fonseca AF, Melfi AJ, Monteiro FA, Montes CR, Almeida VVd, Herpin U (2007): Treated sewage effluent as a source of water and nitrogen for Tifton 85 bermudagrass. Agricultural Water Management 87, 328-336

Dorioz JM, Ferhi A (1994): Pollution diffuse et gestion du milieu agricole: transferts comparés de phosphore et d'azote dans un petit bassin agricole. Water Research 28, 395-410.

Duncan R, Carrow R, Huck M (2000): Effective use of seawater irrigation on turfgrass. USGA Green Section Record 38, 11-17

Duncan RR, Carrow R (2000): Seashore paspalum: The environmental turfgrass. John Wiley & Sons

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK (2005): Global consequences of land use. science 309, 570-574

Fraser LH, Keddy P (1997): The role of experimental microcosms in ecological research. Trends Ecol. Evol. 12, 478-481

Fraser LH, Carty SM, Steer D (2004): A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms. Bioresour. Technol. 94, 185-192

Hu L, Hu W, Deng J, Li Q, Gao F, Zhu J, Han T (2010): Nutrient removal in wetlands with different macrophyte structures in eastern Lake Taihu, China. Ecological Engineering 36, 1725-1732

Huett DO, Morris SG, Smith G, Hunt N (2005): Nitrogen and phosphorus removal from plant nursery runoff in vegetated and unvegetated subsurface flow wetlands. Water Research 39, 3259-3272

Klimkowska A, Kotowski W, van Diggelen R, Grootjans AP, Dzierza P, Brzezinska K (2010a): Vegetation Re-development After Fen Meadow Restoration by Topsoil Removal and Hay Transfer. Restor. Ecol. 18, 924-933

Klimkowska A, Van Diggelen R, Grootjans AP, Kotowski W (2010b): Prospects for fen meadow restoration on severely degraded fens. Perspect. Plant Ecol. Evol. Syst. 12, 245-255

Lee G, Carrow RN, Duncan RR (2005a): Growth and water relation responses to salinity stress in halophytic seashore paspalum ecotypes. Sci. Hortic. 104, 221-236

Lee GJ, Carrow RN, Duncan RR (2005b): Criteria for assessing salinity tolerance of the halophytic turfgrass seashore paspalum. Crop Sci. 45, 251-258

Litaor MI, Eshel G, Sade R, Rimmer A, Shenker M (2008): Hydrogeological characterization of an altered wetland. Journal of Hydrology 349, 333-349

McCarty LB, Miller, B. (2002): Managing Bermudagrass Turf: selection, construction, cultural practices and pest management strategies. Ann Arbor Press, Chelsea

Menzel C, Broomhall P (2006): Response of tropical turfgrasses to recycled water in southern Queensland. Animal Production Science 46, 1645-1652

Nogueira SF, Pereira BFF, Gomes TM, de Paula AM, dos Santos JA, Montes CR (2013): Treated sewage effluent: Agronomical and economical aspects on bermudagrass production. Agricultural Water Management 116, 151-159

Peacock CH, Lee DJ, Reynolds WC, Gregg JP, Cooper RJ, Bruneau AH (2004): Effects of salinity on six bermudagrass turf cultivars. In: Nektarios PA (Editor), Proceedings of the 1st International Conference on Turfgrass Management and Science Sports Fields. Acta Horticulturae. International Society Horticultural Science, Leuven 1, pp. 193-197

Pistocchi C, Guidi W, Piccioni E, Bonari E (2009): Water requirements of poplar and willow vegetation filters grown in lysimeter under Mediterranean conditions: Results of the second rotation. Desalination 246, 137-146

Pistocchi C, Silvestri N, Rossetto R, Sabbatini T, Guidi M, Baneschi I, Bonari E, Trevisan D (2012): A Simple Model to Assess Nitrogen and Phosphorus Contamination in Ungauged Surface Drainage Networks: Application to the Massaciuccoli Lake Catchment, Italy. Journal of Environmental Quality 41, 544-553

Polomski RF, Bielenberg DG, Whitwell T, Taylor MD, Bridges WC, Klaine SJ (2007): Nutrient recovery by seven aquatic garden plants in a laboratory-scale subsurface-constructed wetland. Hortscience 42, 1674-1680

Polomski RF, Taylor MD, Bielenberg DG, Bridges WC, Klaine SJ, Whitwell T (2009): Nitrogen and Phosphorus Remediation by Three Floating Aquatic Macrophytes in Greenhouse-Based Laboratory-Scale Subsurface Constructed Wetlands. Water Air and Soil Pollution 197, 223-232

Rogers K, Breen P, Chick A (1991): Nitrogen removal in experimental wetland treatment systems: evidence for the role of aquatic plants. Research Journal of the Water Pollution Control Federation, 934-941

Schipper LA, McLeod M (2002): Subsidence rates and carbon loss in peat soils following conversion to pasture in the Waikato Region, New Zealand. Soil Use Manage. 18, 91-93

Silvan N, Vasander H, Laine J (2004): Vegetation is the main factor in nutrient retention in a constructed wetland buffer. Plant and Soil 258, 179-187

Soldat DJ, Petrovic AM (2008): The fate and transport of phosphorus in turfgrass ecosystems. Crop Sci. 48, 2051-2065

Tanner C (2001): Plants as ecosystem engineers in subsurface-flow treatment wetlands. Water Science & Technology 44, 9-17

Tanner CC, Clayton JS, Upsdell MP (1995): Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands—II. Removal of nitrogen and phosphorus. Water Research 29, 27-34

Tiemeyer B, Frings J, Kahle P, Kohne S, Lennartz B (2007): A comprehensive study of nutrient losses, soil properties and groundwater concentrations in a degraded peatland used as an intensive meadow - Implications for re-wetting. Journal of Hydrology 345, 80-101

Van der Molen DT, Breeuwsma A, Boers P (1998): Agricultural nutrient losses to surface water in the Netherlands: impact, strategies and perspectives. Journal of Environmental Quality 27, 4-11

Verhoeven JTA, Setter TL (2010): Agricultural use of wetlands: opportunities and limitations. Ann. Bot. 105, 155-163

Volterrani M, Grossi N, Lulli F, Gaetani M (2008): Establishment of warm season turfgrass species by transplant of single potted plants. In: Stier JC, Han L , Li DY (Editors), Proceedings of the Iind International Conference on Turfgrass Science and Management for Sports Fields. Acta Horticulturae, pp. 77-84

Wang D, Dorioz JM, Trevisan D, Braun DC, Windhausen LJ, Vansteelant JY (2004): Using a landscape appraoch to interpret diffuse phosphorus pollution and assist with water quality management in the basin of Lake Champlain (Vermont) and Lac Léman (France) pp 15- 189 In T.O. Manley P.L. Manley and TB Mihuc (eds.) Lake Champlain in the New Millennium. Kluwer Academic Plenim Publisher New york

Wichtmann W, Couwenberg J (2013): Reed as a Renewable Resource and Other Aspects of Paludiculture. Foreword. Mires and Peat 13

Withers PJA, Lord EI (2002): Agricultural nutrient inputs to rivers and groundwaters in the UK: policy, environmental management and research needs. Science of the Total Environment 282, 9-24

Zak D, Gelbrecht J, Zerbe S, Shatwell T, Barth M, Cabezas A, Steffenhagen P How helophytes influence the phosphorus cycle in degraded inundated peat soils – Implications for fen restoration. Ecological Engineering

Zak D, Gelbrecht J, Steinberg CEW (2004): Phosphorus retention at the redox interface of peatlands adjacent to surface waters in northeast Germany. Biogeochemistry 70, 357-368

Figure Captions

Fig. 1 An overview of the mesocosms used in the experiment

Fig. 2 The recovery rate (difference between the total nutrients supply and plant uptakes at mesocosm level) for N and P. The vertical bars represent the standard deviation.

Table

Tab.1 Mean values of the parameters analyzed on the substrates used to fill the pots

	Sand (Sa)	Peat (Pe)
Texture (% clay, silt, sand)	0.49, 0.10, 99.41	5.7, 19.09,75.21
Total Phosphorus (ppm)	274	2837
Particle density (g/cm^3)	3.18	1.97
Organic matter (%)	< 0.1	37.9
pH	8.84	4.76
EC (dS/m)	0.11	0.54

Tab. 2 Results of ONE-Way ANOVA performed on the parameters related to vegetation

	dF	AB	BB	N.T.E.	ET_0	WUE	NCA	PCA	NTA	PTA	NCB	PCB	NTB	PTB	NTT	PTT
Treat	9	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Rep.	2															

Significance: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$
AB (g) = Dry weight of above-ground biomass
BB (g) = Dry weight od below-ground biomass
ET_0 (mm) = evapotranspiration rate
WUE (g/l)= Water Use Efficiency
NCA (mg/g)= N concentration in above-ground tissues
PCA (mg/g) = P concentration in above-ground tissues
NTA (mg) = N uptake of above-ground biomass
PTA (mg) = P uptake of above-ground biomass
NCB (mg/g) = N concentration in below-ground tissues
PCB (mg/g) = P concentration in below-ground tissues
NTB (mg) = N uptake of below-ground biomass
PTB (mg) = P uptake of below-ground biomass
NTT (mg) = N uptake of the total plant
PTT (mg) = P uptake of the total plant

Tab. 3 Results of ONE-Way ANOVA performed on the parameters related to vegetation.

	dF	P-Ols	P-Tot	N-Tot	pH	EC
Treat	11	***	***	***	***	*
Rep.	2					

Significance: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$

P-Ols (ppm): available phosphorus

P-Tot (ppm): total phosphorus

N-Tot (g/kg): total nitrogen

EC ($\mu S \cdot cm^{-1}$): Electrical Conductivity

Tab. 4 Mean separation by orthogonal contrasts of parameters related to vegetation

	Cd vs Pv			Pe vs Sa			Cd-C vs Pv-C		
AB	36.5	33.7		45.5	19.4	***	14.8	11.6	
BB	36.2	55.4	***	43	39.1	***	37.3	43.3	
ETo	297	307		294	208	***	259	249	
WUE	2.2	2.0	***	2.4	1.7	***	3.7	4.3	
NCA	8.7	11.7	***	10.1	10.4		9.3	10.5	
PCA	1.3	1.5		1.7	1.0	*	1.7	2.8	**
NTA	354.5	490.8	**	503.5	237.8	***	136.6	122.0	
PTA	54.7	40.3	*	61.9	18.6	***	24.4	32.1	
NCB	5.9	5.1		6.7	4.7	***	5.4	7.4	**
PCB	1.4	1.4		1.8	0.8	***	2.0	2.0	
NTB	202.2	371.5	***	354.9	184.8	***	202.4	321.7	
PTB	53.9	82.6	**	94.2	29.3	***	74.6	109.2	*
NTT	556.7	862.3		858.4	422.6	**	339	443.7	***
PTT	108.6	122.9	**	156.1	47.9		99	141.3	***

Significance: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$. All the values showed in the table are related to the experimental unit (mesocosm).
AB (g) = Dry weight of above-ground biomass
BB (g) = Dry weight od below-ground biomass
ET_0 (mm) = evapotranspiration rate
WUE (g/l)= Water Use Efficiency
NCA (mg/g)= N concentration in above-ground tissues
PCA (mg/g) = P concentration in above-ground tissues
NTA (mg) = N uptake of above-ground biomass
PTA (mg) = P uptake of above-ground biomass
NCB (mg/g) = N concentration in below-ground tissues
PCB (mg/g) = P concentration in below-ground tissues
NTB (mg) = N uptake of below-ground biomass
PTB (mg) = P uptake of below-ground biomass
NTT (mg) = N uptake of the total plant
PTT (mg) = P uptake of the total plant

Tab. 5 Mean separation by orthogonal interaction contrasts of parameters related to vegetation

	Cd vs Pv : Pe vs Sa					Sa vs Pe : L vs H					Cd vs Pv : PeH vs PeL					Cd vs Pv : SaH vs SaL				
	CdPe	**CdSa**	**PvPe**	**PvSa**		**SaL**	**SaH**	**PeL**	**PeH**		**CdPeH**	**CdPeL**	**PvPeH**	**PvPeL**		**CdSaH**	**CdSaL**	**PvSaH**	**PvSaL**	
AB	42.0	19.2	43.1	19.6		4.2	34.6	24.2	99.3	***	105.3	24.0	93.2	24.4		35.0	3.5	34.3	4.9	
BB	41.7	28.0	58.9	50.3		33.4	44.9	46.0	64.6		45.9	41.9	83.3	50.2	***	31.0	25.0	58.8	41.7	
ETo	360	203	369	213		159	257	284	556	***	547	274	565	293		250	156	263	162	
WUE	2.6	1.7	2.3	1.6		0.7	2.7	2.1	3.8		11.0	5.4	14.6	7.3		7.0	1.3	9.8	2.5	
NCA	8.6	8.8	11.9	12.1		7.9	12.9	8.9	12.7		9.1	7.6	15.1	8.8	**	10.5	7.0	15.3	8.9	*
PCA	1.7	0.8	1.6	0.7		0.8	0.8	1.6	1.0		1.5	1.4	0.8	1.8		1.0	0.9	1.0	0.9	
NTA	381.7	195.7	594.9	279.9		33.6	442.0	215.7	1267.6	***	954.6	181.0	1412.4	214.1	**	367.2	24.3	516.8	42.9	
PTA	65.6	21.7	48.8	12.4	*	3.4	27.7	38.3	122.1	**	161.2	32.8	77.2	43.7	***	34.8	3.3	31.9	4.3	
NCB	5.8	5.0	7.6	4.4	**	4.1	5.3	5.8	7.8		6.8	5.1	8.8	6.6		5.5	4.6	5.1	3.6	
PCB	1.8	0.9	1.8	0.7		0.8	0.8	1.8	1.6		1.4	1.9	1.8	1.7		0.8	1.0	0.7	0.7	
NTB	241.9	142.6	467.9	226.9	*	134.4	235.1	272.8	529.8	*	308.4	214.9	751.1	330.7	**	170.1	115.2	300.2	153.5	
PTB	73.1	25.2	115.3	33.5	*	26.1	32.6	82.5	108.3		66.8	78.0	149.7	87.1	**	26.0	24.3	39.1	27.9	
NTT	623.6	338.3	1062.8	506.8	**	168	677.1	488.5	1797.4	***	1263	395.9	2163.5	544.8		537.3	182.4	817	196.4	**
PTT	138.7	46.9	164.1	45.9		29.5	60.3	120.8	230.5	***	228	110.8	226.9	130.8	**	60.8	27.6	71	32.3	

Significance: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$. All the values showed in the table are related to the experimental unit (mesocosm).
AB (g) = Dry weight of above-ground biomass
BB (g) = Dry weight od below-ground biomass
ET_0 (mm) = evapotranspiration rate
WUE (g/l)= Water Use Efficiency
NCA (mg/g)= N concentration in above-ground tissues
PCA (mg/g) = P concentration in above-ground tissues
NTA (mg) = N uptake of above-ground biomass
PTA (mg) = P uptake of above-ground biomass
NCB (mg/g) = N concentration in below-ground tissues
PCB (mg/g) = P concentration in below-ground tissues
NTB (mg) = N uptake of below-ground biomass
PTB (mg) = P uptake of below-ground biomass
NTT (mg) = N uptake of the total plant
PTT (mg) = P uptake of the total plant

Tab. 6 Mean separation by orthogonal contrasts of parameters related to substrates.

	Cd vs Pv			(PeH +PeL) vs C			Ba vs Veg		
P-Ols	15	12		20	22		25	21	**
P-Tot	1728	1846		2721	2967		3711	2803	***
N-Tot	9.4	9.5		15.8	15.7		16.1	15.8	
pH	6.1	6.2		4.8	4.5	*	4.4	4.7	*
EC	521	506		804	544		418	717	

Significance: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.
P-Ols (ppm): available phosphorus
P-Tot (ppm): total phosphorus
N-Tot (g/kg): total nitrogen
EC ($\mu S \cdot cm^{-1}$): electrical conductivity

Tab. 7 Mean separation by orthogonal interaction contrasts of parameters related to substrates.

	Cd vs Pv : Pe vs Sa				Sa vs Pe : L vs H				Cd vs Pv : PeH vs PeL				Cd vs Pv : SaH vs SaL			
	CdPe	**CdSa**	**PvPe**	**PvSa**	**SaL**	**SaH**	**PeL**	**PeH**	**CdPeH**	**CdPeL**	**PvPeH**	**PvPeL**	**CdSaH**	**CdSaL**	**PvSaH**	**PvSaL**
P-Ols	22	3	19	1	2	2	22	18	20	23	16	21	3	3	2	1
P-Tot	2701	268	2906	257	247	277	2710	2732	2684	2669	2780	2752	266	269	287	226
N-Tot	15.77	0.04	15.88	0.05	0.06	0.03	15.60	16.00	15.60	15.73	16.40	15.47	0.04	0.04	0.03	0.08
pH	4.8	8.1	4.6	8.4	8.2	8.3	4.9	4.8	4	4.7	4.7	4.8	8.3	8.5	8.3	8
EC	715	229	720	187	123	293	695	913	719	759	671	1067	261	112	324	134

Significance: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.
P-Ols (ppm): available phosphorus
P-Tot (ppm): total phosphorus
N-Tot (g/kg): total nitrogen
EC ($\mu S \cdot cm^{-1}$): electrical conductivity

Figure
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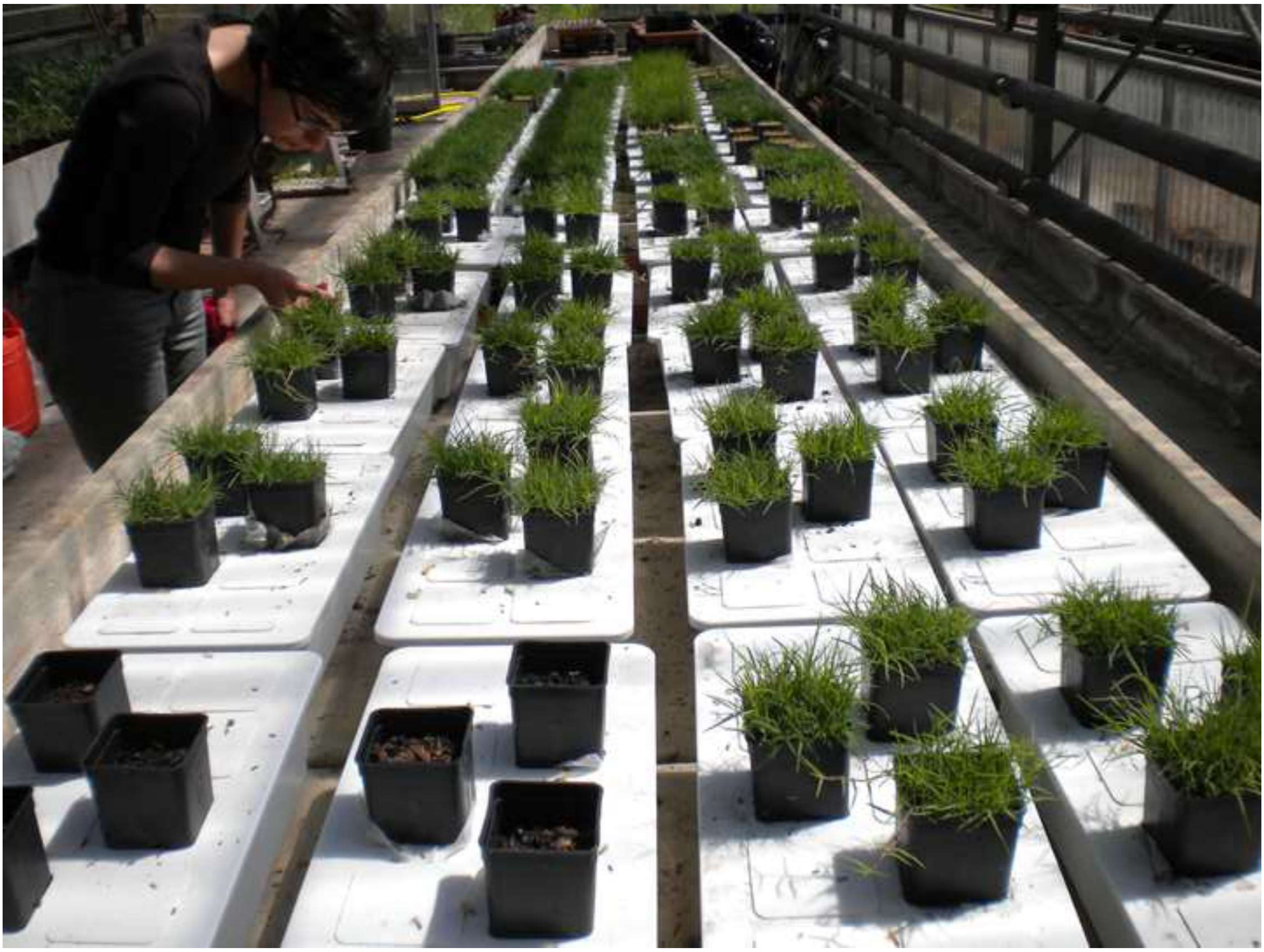
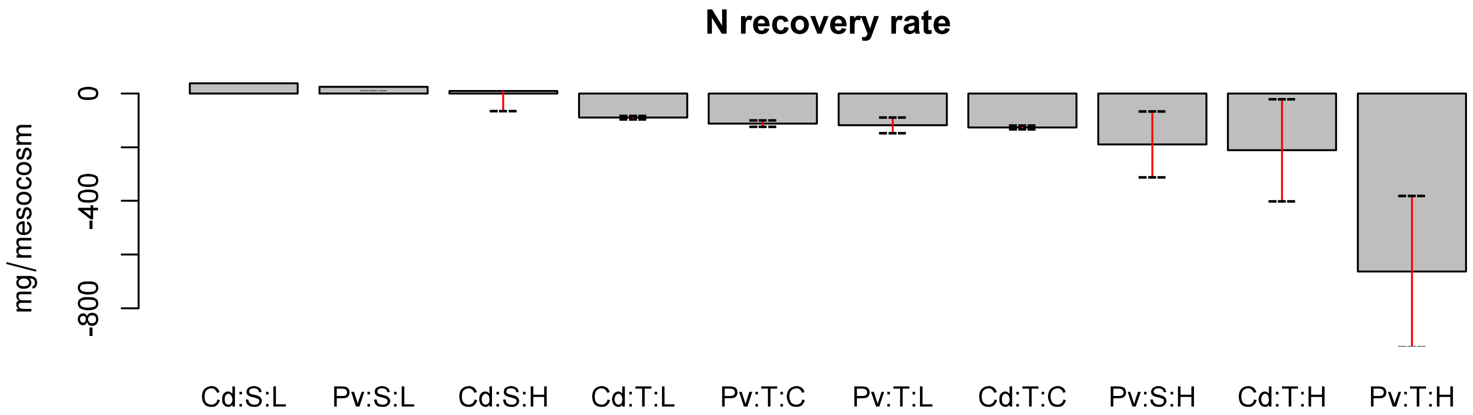


Figure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mg/mesocosm
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-400
-800
Cd:S:L
Pv:S:L
Cd:S:H
Cd:T:L
Pv:T:C
Pv:T:L
Cd:T:C
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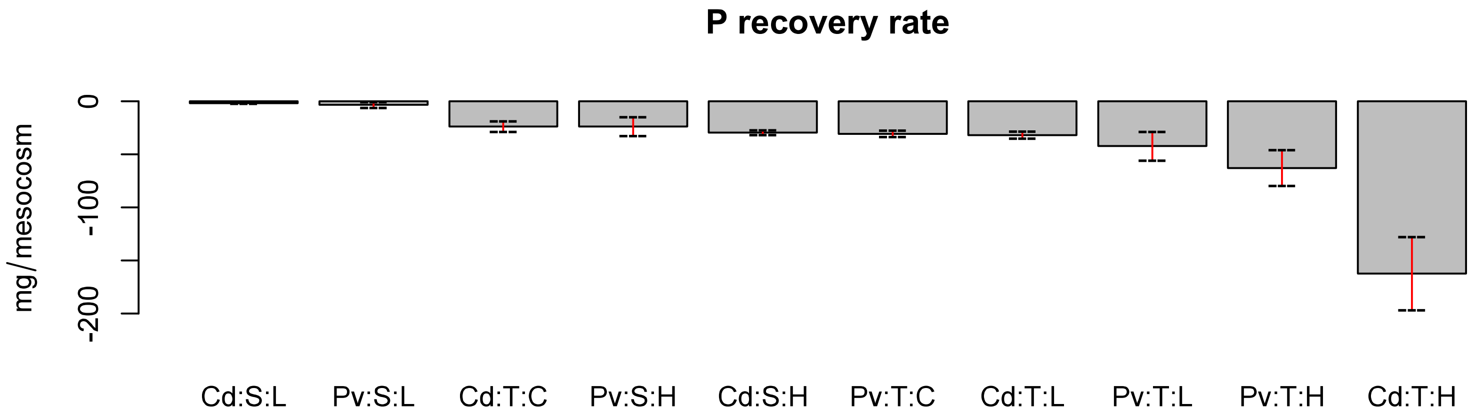
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