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Research Article

Phosphorus Placement Effects on Phosphorous Recovery Efficiency and Grain Yield of Wheat under No-Tillage in the Humid Pampas of Argentina

Pablo Andrés Barbieri,[1,2] Hernán René Sainz Rozas,[1,2,3] Fernanda Covacevich,[2,4] and Hernán Eduardo Echeverría[1,3]

[1] *Estación Experimental Agropecuaria, INTA, C.C.276, Buenos Aires, 7620 Balcarce, Argentina*
[2] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Avenida Rivadavia 1917, C1033AAJ Buenos Aires, Argentina*
[3] *Facultad de Ciencias Agrarias, UNMDP, Unidad Integrada Balcarce, C.C.276, Buenos Aires, 7620 Balcarce, Argentina*
[4] *Instituto de Investigaciones en Biodiversidad y Biotecnología, INBIOTEC-FIBA, Mar del Plata, Argentina*

Correspondence should be addressed to Pablo Andrés Barbieri; barbieri.pablo@inta.gob.ar

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No-till (NT) affects dynamics of phosphorus (P) applied. Wheat response to P fertilization can be affected by available soil P, grain yield, placement, rate, and timing of fertilization. Furthermore, mycorrhizal associations could contribute to improving plant P uptake. Three experiments were used to evaluate P rate (0, 25, and 50 kg P ha^{-1}) and fertilizer placement (broadcasted or deep-banded) effects in NT wheat on P recovery efficiency (PRE) yield and arbuscular mycorrhizal colonization (AMC) which was assessed in one experiment. Fertilization increased dry matter (DM) and accumulated P. Broadcasted P produced lower P accumulation than deep-banded P only at tillering. Phosphorus rate decreased PRE, and placement method did not affect it. Grain yield response was increased by P rate (857 and 1805 kg ha^{-1} for 25 and 50 kg P ha^{-1}, resp.) and was not affected by placement method (4774 and 5333 kg ha^{-1} for broadcasted and deep-banded, resp.). Deep-banded P depressed root AMC compared with broadcast applications. Highest AMC in P broadcasted treatments could help to explain the lack of differences between placement methods. These results indicate that Mollisol have low P retention capacity. Therefore, broadcasted P could be used as an alternative of fertilizer management for NT wheat.

1. Introduction

Most agricultural production in Argentina comes from the Mollisols of the Pampas region; many of these are among the most fertile soils in the world [1]. These soils were developed under grassland from loess predominantly deposited by aeolian processes [2]. In this region Udic and thermic are the prevailing water and temperature regimes [1]. In general, the phosphorus (P) sorption capacity of these soils is relatively low [3, 4].

In this region, among winter crops, spring wheat is the most important. Within the Argentine Pampas Region, the Southeastern Buenos Aires province (Figure 1) represents an important wheat production area, contributing 20% to the national production. This area has an average annual temperature of 13.8°C and an average rainfall of 870 mm, 45% of which occurs during the wheat growing season (June–December). It was reported that approximately in 3 out of 30 yr the rainfall during the early season on a wheat crop (June–September) is lower than the potential evapotranspiration [5].

No-tillage agriculture (NT) has become more widespread in Argentina reaching approximately 67% of the total sowing area in the 2007-2008 growing season [6]. No-tillage produces physical, chemical, and biological changes in the soil, affecting both the dynamics and response of wheat to

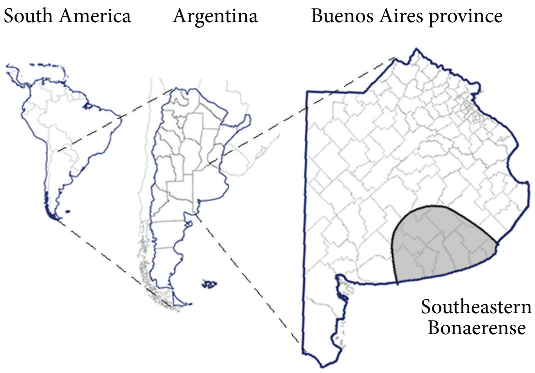


Figure 1: Map of South America indicating Argentina and the shadow area that comprises the Southeastern Buenos Aires.

P fertilization. It was reported that NT increases available P, organic matter (OM) in soil surface horizons [7–9], and soil phosphatase activity [10] and produces changes in roots distribution, leading to greater root density in soil surface horizons [11]. High residue coverage usually increases soil moisture and reduces soil temperature at shallow depths, which can inhibit plant growth and P availability early in the season [12–14]. Guertal et al. [15] reported a low P retention capacity in surface soil under NT. This is likely a consequence of a high concentration of labile P forms (phosphate ions adsorbed with low retention energy), which saturates the P fixation sites, and of the greater OM concentration [8, 16] which would contribute to diminish P retention, due to a negative relationship between OM and P retention [17].

Soil environment, as well as the plant physiological conditions, can be greatly changed through tillage; therefore soil microbial populations could be also affected. It is well accepted that tillage directly affects all types of arbuscular mycorrhizal fungi (AMF) propagules (spores, colonized root fragments, and free hyphae of bulk soil) through different mechanisms acting together: (i) disruption of the hyphal network; (ii) dilution of the propagule-rich topsoil; and (iii) accelerated root decomposition [18]. Some reports have shown that tillage can reduce either AMF spore density [19] or root mycorrhizal colonization of crops and the potential for P uptake [20]. Damage to the hyphal network by tillage can reduce AMF growth and root colonization due to death or reduced infectivity of the hyphal fragments compared with intact networks and by detaching them from the host plants [21, 22]. Kabir et al. [19, 23] have shown that disruption of hyphae through soil disturbance reduces infectivity and viability of AMF and thus depresses mycorrhizal potential. They showed that fallow had negative effects on the abundance of metabolically active hyphae were concurrent with the decrease in nutrient content by the corn plant. However the time between the disturbance and the viability of the hyphae is an aspect that should be considered. Kabir et al. [19] pointed that when most of the fallow periods (except 30 d of fallow) were applied, disturbance of the soil did not always reduce root mycorrhizal colonization. However, because mycorrhizal communities are site specific and each AMF species can be affected in several ways by different agricultural management practices, generalization is difficult. For example, Menéndez et al. [24] found that tillage reduced spore number and AMF species diversity in agricultural soil of the Pampas region, whereas Schalamuk et al. [25] reported that tillage did not affect biodiversity of a soil from a wheat monoculture in the same region. While there are conflicting reports regarding the tillage and AMF, there are very few reports in relation to the location of P and mycorrhizae.

Land use and yield average of main crops were remarkably increased in the last 20 years in the Pampas Region [26]. This situation has generated a progressive decrease of nutrient such as P, and, therefore, a generalized crop response to P application. In the Southeastern Buenos Aires province it has been reported that values of Bray-P [27] lower than 16 mg kg^{-1} soil decreased wheat grain yield [28]. In this area, greater P use efficiency was determined for banded (5 cm beside and below the seeds) compared to broadcasted fertilization at sowing under conventional tillage, mainly in soils with low available P levels (7 to 12 mg kg^{-1}) [29, 30]. However, under NT, Bordoli and Mallarino [31] found no differences in maize grain yield between broadcasted before sowing (3 month) versus deep-banded (5–10 cm below surface) P fertilization. Similar results were reported by Bordoli et al. [32] for wheat under NT in a clay texture soil and low levels of available P (approximately 8 mg kg^{-1}). Response of crops to P placement may also be affected by the amount and distribution of rainfall. In subhumid or semiarid regions with higher probability of rain deficit, P uptake from broadcasted P fertilization could be restricted as consequence of low water content in the topsoil. This also produces changes in the growth, activity, and distribution of root with depth [33].

For high productivity crops in these conditions, formation of depletion zone (root-soil interface) of nutrients (mainly P) around the roots could be limiting for crop production. Although fertilization is the common used practice to minimize nutrient depletion, it is possible that contribution of AMF could contribute to P uptake beyond the rhizosphere depletion zone [34–36]. However, fertilization may negatively affect the formation of the symbiosis. Covacevich et al. [37] showed that the AMC of wheat roots was modulated (depressed by high P concentration) by current local soil available P as a result of fertilization with inorganic P source (superphosphate (SP)) but not by plant P status. Moreover, Covacevich et al. [30] found that fertilizer placement also affects indigenous AMC of wheat crops because deep-banded SP depresses indigenous mycorrhizal formation compared with broadcasted applications under tillage. However, the knowledge about the effect of P placement on wheat mycorrhiza under NT is still poor.

In the Southeastern Buenos Aires province little information exists about wheat response to broadcasted P under NT. Phosphorous fertilization broadcasted before sowing (3 months) is a practice that saves time and work at sowing, diminishes soil compaction, and eliminates operative disadvantages, mainly under wet climate conditions, that normally occur during wheat sowing period. Another advantage for broadcasted P is the decrease of the horizontal P variability caused by banded fertilization, which often increases the testing error of soil P availability [38]. Nevertheless, there are environmental risks associated with leaving P on the soil surface, P may be lost from soil through leaching and surface runoff. Especially in soils with low P-retention properties and/or significant preferential flow pathways (e.g., cracking clay soils) [39].

Efficient fertilizer management should combine rate, timing, placement, and source in a way that optimizes crop yield and quality, minimizing nutrient losses to the environment [40]. Because P fertilization is an important cost of crop production, there is a need to develop agricultural systems based on efficiently meeting crop requirements without applying excess fertilizer. This will require a detailed understanding of the processes governing soil P cycling and availability in which mycorrhizal symbiosis may play a significant role. Our hypothesis was that, in Mollisols under NT in the Southeastern Buenos Aires province, fertilizer P placement does not affect wheat grain yield, P uptake, and recovery efficiency of wheat crop. Our research objective was to evaluate the effects of P rate and placement in wheat crop under NT on P uptake, grain yield, and PRE. In addition, we evaluate arbuscular mycorrhizal colonization at a site to know whether the mycorrhizal symbiosis could help to explain some results obtained.

2. Materials and Methods

The study was conducted at three experimental sites: Tandil, Necochea, and Balcarce (Figure 1). Soils at Tandil and Mar del Plata are Typical Argiudolls, with loam texture in the surface layer (0 to 25 cm), a loam to clay-loam texture in subsurface layers (25 to 110 cm), and a sandy-loam texture below 110 cm depth (C horizon). The soil in Balcarce was a Petrocalcic Paleudoll, which presents discontinuous layers of Petrocalcic horizon below 0.8 m and greater clay contents at subsurface layers than Typical Argiudolls. These sites had 1% slope and therefore no erosion is present, NT was initiated more than 7 yr previously, and the entire crop rotation is corn, soybean, and double crop wheat/soybean. Some characteristics of management practices, cultivars, and the properties of the surface soil of the experimental sites are presented in Table 1.

At Tandil and Necochea the experimental design was randomized complete blocks with a factorial (2×2) treatment arrangement of P rate (25 and 50 kg P ha^{-1}) and placement method (P broadcasted 3 months before sowing) and P banded (5 cm beside and below the seeds sowing). Two reference treatments (0 and 150 kg P ha^{-1} broadcasted) were also added for analysis of P response, and data were analysed using design randomized complete block (P rate of 25 and 50 kg P ha^{-1} were an average of banded and broadcast P application). Phosphorous response was evaluated using 0, 25, 50, and 150 kg P ha^{-1}. In Balcarce, the experimental design was a randomized complete block, and treatments were 0 and 25 kg of P ha^{-1} broadcasted or deep-banded. In all sites, the experimental units were 75 m^{-2} (5 m × 15 m). The P source was diammonium phosphate (18-46-0). Sulphur and N fertilizers were applied according to local recommendations at the rate of 10 kg/ha S (as calcium sulfate) and 150 kg N/ha (as urea). The urea was applied at tillering [41], while S was applied at showing. Weeds were controlled with applications of metsulfuron methyl (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amine]carbonil]amine]sulfonil] benzoate) at 6 g a.i. ha^{-1} plus 2,4-D (2,4-dicholorophenoxyacetic acid) at 0.5 kg a.i. ha^{-1}. When necessary, insects were controlled by application of deltamethrin [(S)-Cyano-(3-phenoxyphenyl)-methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate at 5 g a.i. ha^{-1}.

At Tandil and Necochea 0–10 and 10–20 cm soil samples were collected at tillering [42] ($Z = 22$) and flowering ($Z = 60$) and Bray-P was determined. Only at Necochea at $Z = 22$ and $Z = 60$, 4 soil samples per plot (2 in the row and 2 inter-row) were collected for AMC determination (10 cores of 5 cm diameter and 20 cm depth). Each soil core was divided in 2 subfractions of 0–10 and 10–20 cm; roots of each soil depth were separated from soil, washed to remove soil particles, collected on a sieve (2 mm) cut thoroughly, mixed, and stained according to the Phillips and Hayman [43] modified method. Briefly roots were cleared with KOH (10%, 30 min, 100°C), acidified with HCl (0.1 N, 2 min), and stained with Trypan Blue (0.05%, 5 min, 100°C) in lactoglycerol (lactic acid, glycerol, distilled water 1 : 1 : 1). The occurrence of mycorrhizal colonization was assessed by microscopic examination (40x and 100x) of the stained roots system. A segment was considered colonized if it contained arbuscules, coils plus hyphae, and/or vesicles. Colonization was assessed using the Trouvelot et al. [44] method that allowed the simultaneous evaluation of the intensity of AMC and the proportion of arbuscules of roots.

Soil P retention capacity [45] was determined in 0–10 and 10–20 cm soil samples from the Necochea site that were collected at sowing from the control treatment (0P). Inorganic P in the supernatant was determined by colorimetry [46]. P retention was analyzed using a split-plot in a randomized complete blocks design with three replications, where the main plot was the soil depth (0–10 and 10–20 cm) and the subplot was the three concentrations of P added (0, 60, and 120 ug P per g soil). Percentage of P retention was calculated as 100 − ((Bray P60 or 120 − Bray P0)/60 or120) ∗ 100 using Bray-P [27], and percentage of P recovery was calculated as ((Bray P60 or 120 − Bray P0)/60 or 120) ∗ 100.

For all sites, aboveground DM accumulation at tillering, first node, flowering, and physiological maturity were determined by harvesting an area of 1.2 m^2. At maturity, the harvest was done by cropper and grain yield was determined by harvesting an area of 9.6 m^2 and expressed to 140 g kg^{-1} grain moisture content. At Tandil and Necochea, plant P accumulation [47] was measured at tillering ($Z = 22$), first node ($Z = 31$), flowering ($Z = 60$), and physiological maturity. Grain P and straw content were determined separately. At physiological maturity, PRE in DM (grain + straw) and grain were calculated as follows: (P content in DM or grain at the fertilized treatments − P content in DM or grain at control (0P))/P rate.

Treatment effects were evaluated by analysis of variance using the SAS software [48]. Interactions among sources of variations (for all sites, harvest and evaluated parameters)

Table 1: Some characteristics of the soil surface at Tandil, Necochea, and Balcarce experimental sites.

Site	OM %	AP mg kg^{-1}	pH	Texture	PC	Tillage	Cultivar	Row spacing (cm)	Seeding date	Seeding rate kg ha^{-1}	Harvest date
Tandil	5.5	8.9	6.3	Loam	Sb	No-till	Prointa Isla Verde	19.0	August 15	140	December 27
Necochea	5.7	13.9	6.0	Loam	Sb	No-till	Klein Dragon	19.0	August 12	140	December 28
Balcarce	4.3	9.9	6.2	Loam	Sb	No-till	Baguette 19	17.5	July 18	120	December 23

OM: organic matter [66]; AP: available phosphorus (Bray and Kurtz 1945) [27]; PC: previous crop; Sb: soybean.

Table 2: Monthly rainfall (mm), mean minimum temperature (°C), mean maximum temperature (°C), and crop evapotranspiration (CET) during wheat growing season at Tandil, Necochea, and Balcarce experimental sites.

	Tandil				Necochea				Balcarce			
	Rainfall	CET	Temp. Min	Temp. Max	Rainfall	CET	Temp. Min	Temp. Max	Rainfall	CET	Temp. Min	Temp. Max
	mm		°C		mm		°C		mm		°C	
July	41	22	3.1	12.3	131	27.1	2.6	12.3	13	29	0.7	11.3
August	132	36	5.9	14.0	55	37.9	3.1	13.3	25	41	1.4	12.1
September	58	35	5.0	15.3	17	50.4	4.9	16.2	183	58	7.6	17.2
October	175	72	9.1	20.5	100	91.5	7.8	20.0	117	92	9.9	20.1
November	143	76	11.4	22.1	76	111.5	9.2	21.8	45	113	7.4	21.9
December	56	84	13.4	25.5	118	136.8	11.9	24.0	35	161	11.8	27.3

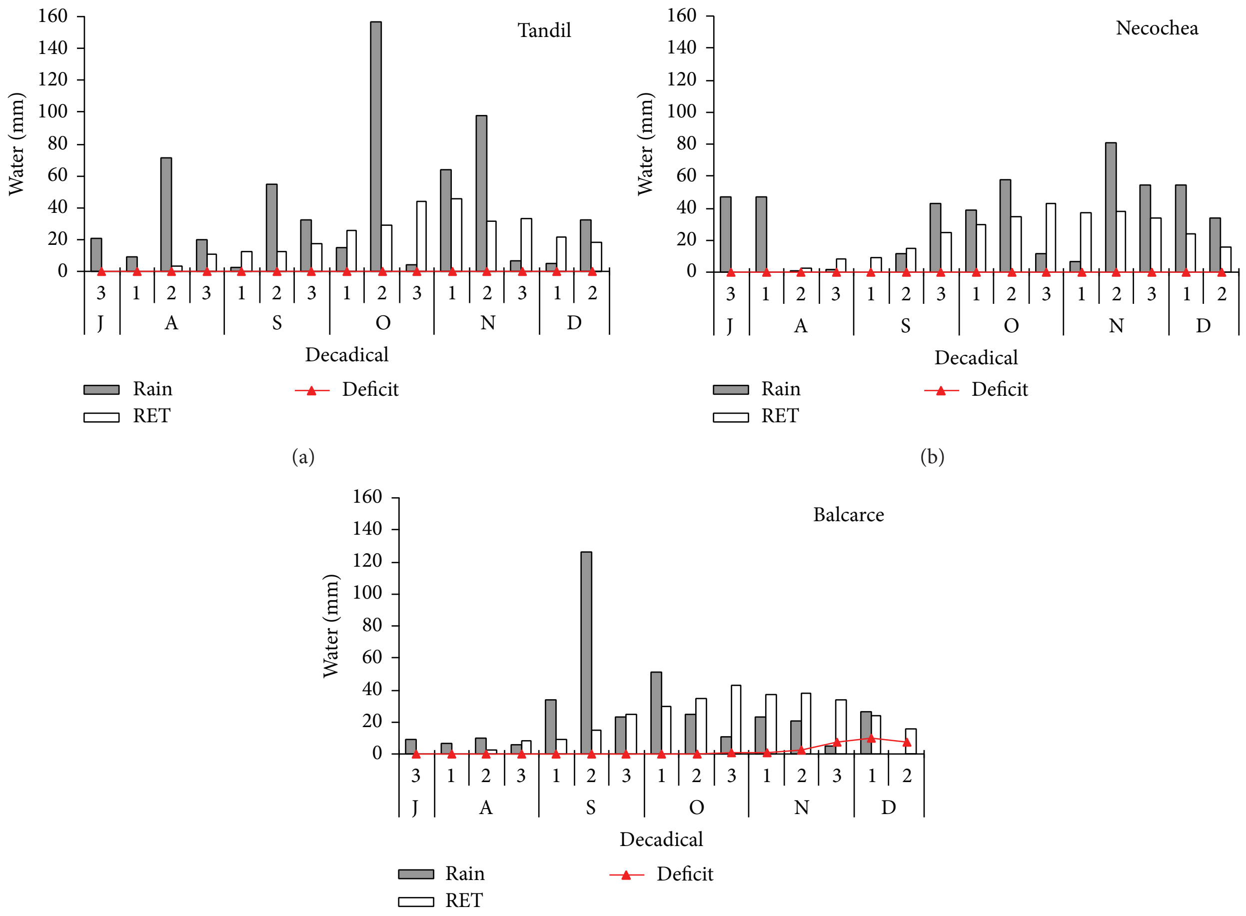
were not significant. Thus plant and soil data are shown as affected by pure treatments. The AMC data were analysed as independent treatment at each soil depth. Differences in means for DM, P uptake, PRE, grain yield, P retention in soil, and AMC were compared with the Duncan multiple range test ($P < 0.05$). All presented data are means of untransformed values.

3. Results and Discussion

3.1. Environmental Characterization of Experiments. Some characteristics of rainfall, crop evapotranspiration [49], and temperatures (minimum and maximum) during the wheat growing season are present in Table 2. Accumulated rainfall during June–December was 617 and 506 mm in Tandil and Necochea, respectively. Water balances for the wheat crops during growing seasons were calculated taking into account soil water storage capacity [50] (Figure 2). Water availability did not limit wheat growth or grain yields in these sites, and in consequence high grain yield were achieved (4970 and 6160 kg ha^{-1} on average of P rate at Tandil and Necochea, resp.). At Balcarce, accumulated rainfall during June-December was 447 mm and water stress occurred after flowering (later November-December) which may have affected grain-filling and in consequence grain yield. Additionally, low temperatures in November caused frost damage, affecting wheat grain yield (3790 kg ha^{-1} averaged over P rates).

3.2. Aboveground Dry Matter Accumulation. Phosphorus rate and placement affected DM accumulation (Table 3). For all sites, P fertilization increased DM accumulation indicating that soil P availability affected wheat growth (Table 3). Available soil P at sowing was 8.9, 13.9, and 9.9 mg kg^{-1} in Tandil, Necochea, and Balcarce, respectively, and these values were lower than the P response threshold of 16-17 mg kg^{-1} [28]. Phosphorus rate of 150 kg ha^{-1} produced a greater DM accumulation than 25 and 50 kg P ha^{-1} only in Necochea at tillering and at first node (Table 3). Placement method affected wheat crop growth mainly during early stages. At Balcarce, the same tendency was observed, although the difference between placement methods was not statistically significant (Table 3). Dry matter accumulation was not affected by fertilizer placement at flowering and physiological maturity (Table 3). These results indicate that initial soil P availability in the banded treatment was greater than in the broadcasted treatment. This agrees with data reported by Mallarino et al. [51] and Borges and Mallarino [14] for maize and soybean in Iowa, respectively. These authors determined higher DM accumulation and P uptake at initial growth stages for banded P applications than for broadcast application (3 or 4 months before sowing).

3.3. Phosphorus Accumulation in Aboveground Dry Matter and Phosphorus Recovery Efficiency. At Tandil and Necochea, DM P accumulation was increased by P rate, and, for all stages, plots fertilized with 150 kg P ha^{-1} produced greater P accumulation than the 25 and 50 kg P ha^{-1} treatments (Table 4). Nevertheless, placement method affected accumulated P only at tillering (Table 4). It is probable that low soil temperatures typically observed at initial stages of the growing season, which are likely lower under NT [52], could have diminished P diffusion to roots, root growth, and P uptake from broadcast P [52]. For both sites, P content in grain increased with P rate but was not affected by placement method (Table 3). Harvest P index was not affected by P rate or fertilization placement method, and it was on average 73%; this value is similar to that reported by García and Berardo [28].



(a) (b) (c)

Figure 2: Precipitation, real evapotranspiration (RET), and water deficit during wheat growing season at Tandil, Necochea, and Balcarce sites.

Plant P recovery (grain plus residues) efficiency at physiological maturity decreased with P rate and was not affected by fertilization placement method (Figure 3). A similar observation was obtained for PRE in grain (Figure 3) with higher values than those reported by Halvorson and Havlin [53], which oscillated from 4 to 5.4% for rates 34 and 68 kg P ha^{-1}, in winter wheat under NT grown in calcareous soils with pH 7.8. These differences indicate that soils (Typical Argiudolls) of Southeastern Buenos Aires province with subacid pH may have a low P retention capacity. These results are similar to those reported by Berardo et al. [54].

3.4. Grain Yield. Tandil and Necochea had higher grain yields than Balcarce (Figure 4) likely due to more favourable climatic conditions. At all sites grain yield was increased by P rate and was not affected by fertilization placement method (Figure 4). At Tandil site only the 25 kg P ha^{-1} rate did not increase grain yield compared to control ($P = 0.06$) while in Necochea site the maximum grain yield was reached with 25 kg P ha^{-1}. Grain weight was not affected by P rate (data not shown) indicating that P availability affected the grain number, which agrees with that reported by other authors [55, 56]. Low P availability affects wheat growth by reducing the rate of emergence and leaf expansion, the number of tillers, and the rate of photosynthesis per unit leaf area [57, 58], resulting in reduced interception and conversion efficiency of incident radiation. Lázaro and Abbate [56] reported that P stress reduces the grain number by a lower production of photoassimilates during spike growth period. Similar grain yield for broadcast and banded P suggests that reduced crop growth rates, caused by early P deficiency, did not affect the crop growth rate during the critical period for kernel set.

The similar yield for broadcasted P application compared to deep-banded P agrees with that reported by Bordoli and Mallarino [31] and Borges and Mallarino [14], for corn and soybean under NT, respectively. However, these results did not agree with those reported for wheat under CT by Berardo et al. [29] and Covacevich et al. [30], who found differences between banded and broadcast P applications when soil P-Bray was less than 12-13 mg kg^{-1}. This suggests that the tillage system could affect the crop response to the P placement method. Halvorson and Havlin [53] for winter wheat under NT reported greater grain yield for banded P application than broadcast mainly at low P rate in soils with pH of 7.8. However, our results suggest similar grain yield response between placement regardless of the P rate and initial available soil Bray-P content.

Table 3: Aerial dry matter accumulation affected by P rate and placement method at Tandil, Necochea, and Balcarce experimental sites.

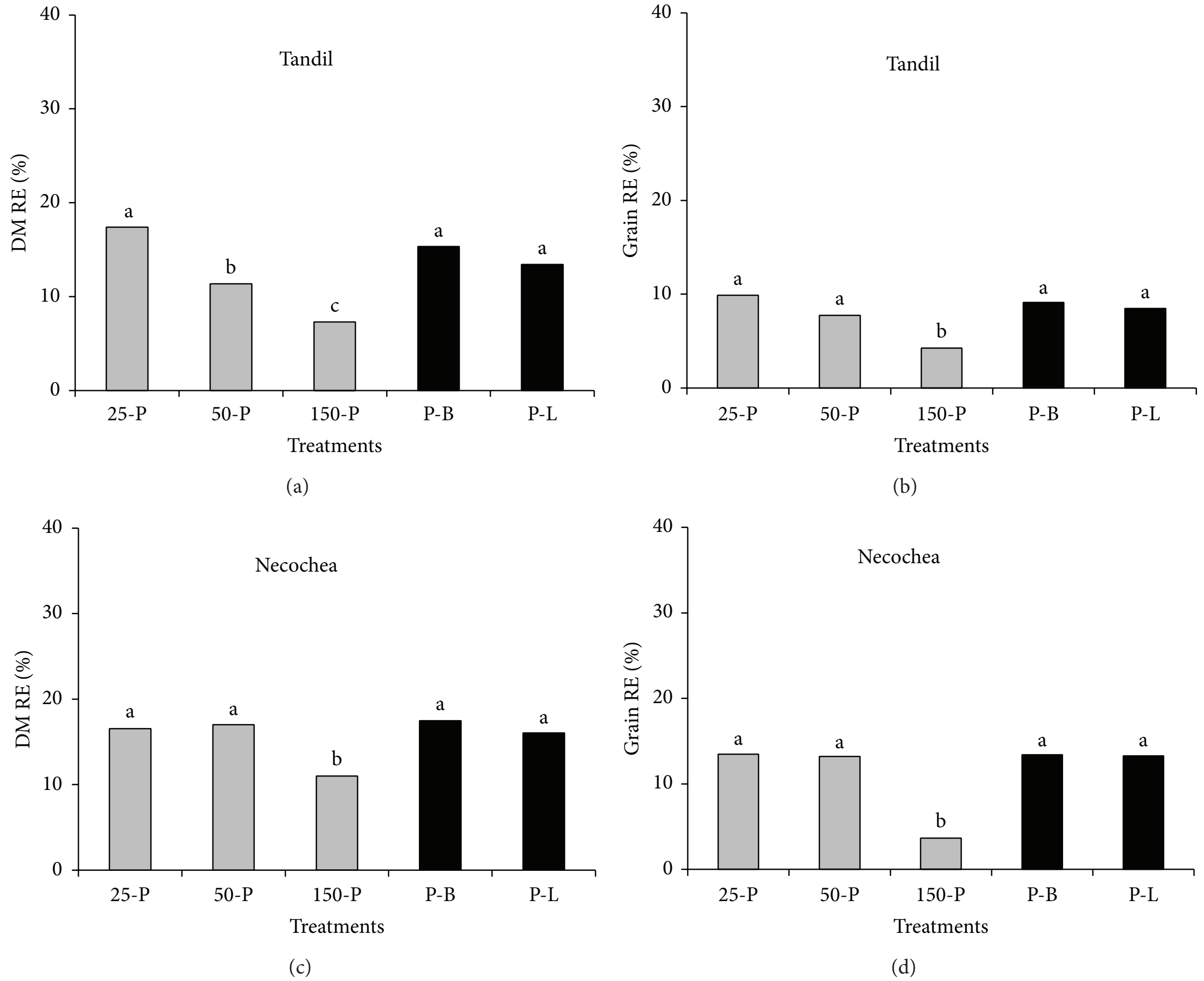
	Tillering	First node	Flowering	PM
		Tandil		
P response			kg ha^{-1}	
0	218 a	1262 b	6175 a	10958 a
25	303 a	1961 a	6668 a	13438 a
50	320 a	2137 a	7669 a	13346 a
150	349 a	2432 a	7802 a	14111 a
Broadcast (*B*)	282 b	1808 a	7136 a	13739 a
Deep-banded (*L*)	341 a	2200 a	7201 a	13046 a
$B \times L$	ns	ns	ns	ns
		Necochea		
P response			kg ha^{-1}	
0	288 d	2939 c	8795 b	15306 a
25	495 c	4519 b	10377 a	16229 ab
50	587 b	4706 b	11254 a	16917 ab
150	693 a	5028 a	10602 a	17247 b
Broadcast (*B*)	458 b	4402 b	10501 a	16549 a
Banded (*L*)	624 a	4824 a	11129 a	16597 a
$B \times L$	ns	ns	ns	ns
		Balcarce		
P response			kg ha^{-1}	
0-P	478 b	1557 b	6838 a	9188 b
25-P-broadcast	761 a	1987 a	7724 a	11103 a
25-P-banded	932 a	2171 a	7267 a	12146 a

Means in the same column followed by the same letter are not significantly different from each other based on the Duncan test (0.05). Phosphorus response is compared using 0, 25, 50, and 150 P rates. Phosphorus placement method is only compared at 25 and 50 P rates. PM: physiological maturity; ns: not significant.

Table 4: Accumulated phosphorous in aerial dry matter. P content in grain and phosphorous harvest index (PHI) at Tandil and Necochea experimental sites.

	Tillering	First node	Flowering	PM	P Grain	PHI
			Tandil			
P response			kg P ha^{-1}			%
0	0.42 b	3.63 c	16.70 b	14.55 b	12.74 c	0.87 a
25	0.96 ab	7.05 bc	16.42 b	19.06 b	15.17 bc	0.80 a
50	1.24 ab	8.52 b	20.10 ab	20.13 ab	16.57 ab	0.82 a
150	1.61 a	14.8 a	24.31 a	25.32 a	19.08 a	0.76 a
Broadcast (*B*)	0.88 a	7.74 a	18.92 a	19.93 a	16.00 a	0.81 a
Banded (*L*)	1.32 a	7.84 a	17.60 a	19.27 a	15.74 a	0.81 a
$B \times L$	ns	ns	ns	ns	ns	ns
			Necochea			
P response			kg P ha^{-1}			%
0	0.72 d	6.66 c	14.64 c	18.15 c	14.07 c	0.78 a
25	1.83 c	10.18 cb	18.03 bc	22.34 bc	17.47 b	0.78 a
50	2.49 b	12.78 b	21.25 b	26.71 b	19.60 a	0.78 a
150	3.12 a	16.88 a	26.39 a	34.73 a	20.70 ab	0.58 b
Broadcast (*B*)	1.59 b	12.04 a	19.72 a	24.57 a	18.93 a	0.77 a
Banded (*L*)	2.65 a	10.93 a	19.66 a	24.47 a	19.24 a	0.79 a
$B \times L$	ns	ns	ns	ns	ns	ns

Means in the same column followed by the same letter are not significantly different from each other based on the Duncan test (0.05). Phosphorus response is compared using 0, 25, 50, and 150 P rates. Phosphorus placement method is only compared at 25 and 50 P rates PM: physiological maturity; ns: not significant.

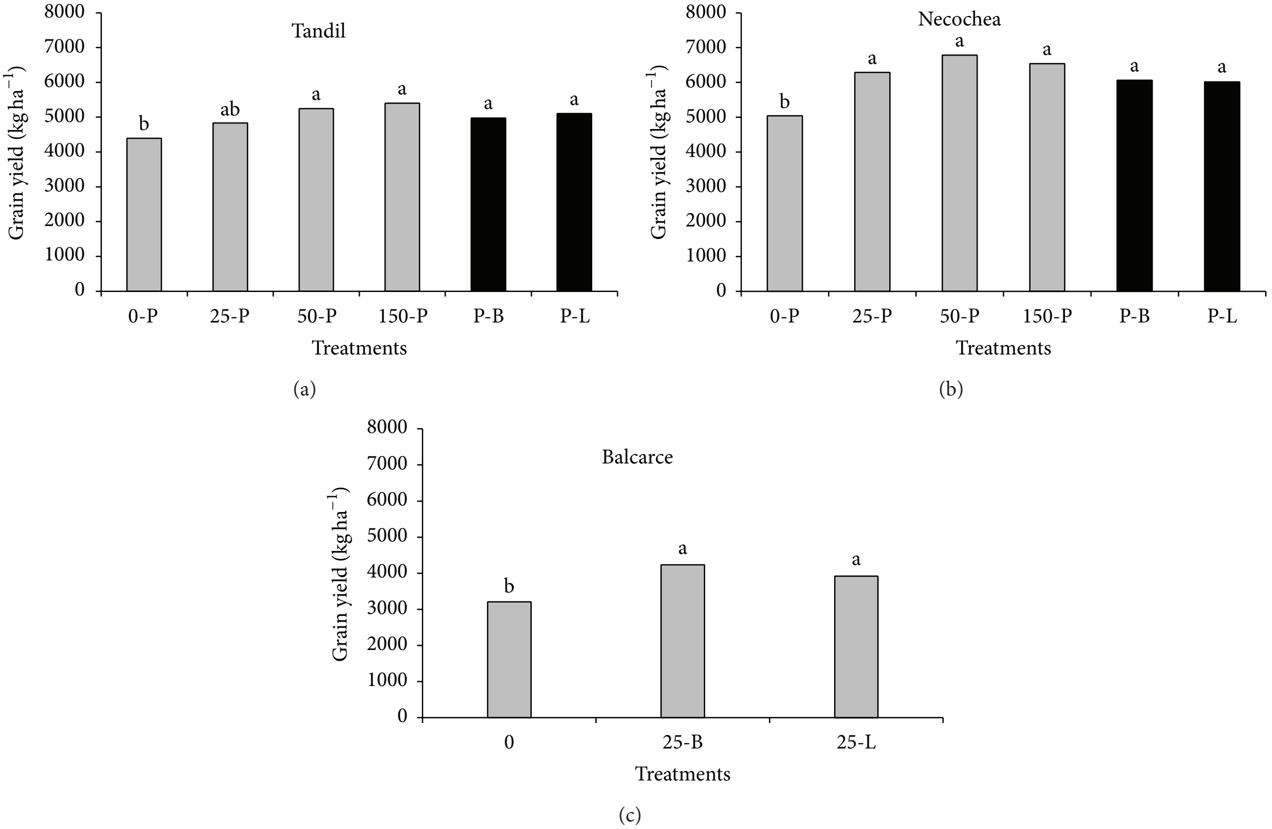


(a) (b) (c) (d)

FIGURE 3: Phosphorus recovery efficiency (PRE) in dry matter (DM = grain + residues) and grain of NT wheat as affected by placement method and P rate. Means followed by the same letter are not significantly different from each other based on the Duncan test (0.05). Phosphorus response is compared using 25, 50, and 150 P rates (gray). Phosphorus placement method is only compared at 25 and 50 P rates (black). P-B; phosphorus broadcasted. P-L: phosphorus banded at sowing.

3.5. Effect of Fertilization Placement Method on Bray-P Content and Phosphorus Recovery. No significant interaction between P rate and placement method was found for Bray-P content at 0–10 cm and 10–20 cm soil depths. Phosphorous fertilization increased Bray-P concentration in the 0–10 cm and 10–20 cm soil depths. Highest soil P contents were found within the top 10 cm of the soil profile when compared to the 10–20 cm depth (Figure 5). Our results are also in accordance with the P decrease throughout the soil profile of soils from the Southeastern Buenos Aires province [59]. Moreover, Culleton and Murphy [60] have also shown that the slow P mobility through the soil normally does not surpass 10 cm of soil depth.

Fertilization placement method did not affect soil Bray-P concentration in 0–10 cm and 10–20 cm soil depths (Figure 5). The greater Bray-P concentration in the 10–20 cm soil depth in fertilized treatments compared to the control (0-P) would suggest that broadcast P moved up to 10–20 cm into the soil. Phosphorus retention was affected by P rate and sampling depth (Table 5). Phosphorus rate at 60 (μg P per g soil) shows a greater retention than 120 (Table 5), indicating that the P fixation by the soil is more important at low P rates of this nutrient. Lower retention of P added was observed in the 0–10 cm depth compared to the 10–20 cm soil depth (Table 5); similar results were reported for soils under long term NT by Guertal et al. [15]. These authors determined that P retention varied from 8 to 14%, 19 to 32%, and 34 to 57% of P added, for 0–2 cm, 6–8 cm, and 16–18 cm soil depths. Possible mechanisms that explain this behaviour are high saturation of P retention sites and the high OM content of surface soil [15]. The great increase of Bray-P in the first 0-10 cm and movement of P in the soil horizon would indicate low P retention of surface soil, allowing that fertilizer P remains in labile mineral forms or that remain as phosphate in solution [15]. Low P retention under non-labile forms might be a consequence of acidic pH, low clay content (1 : 1), low interchangeable Al, low Al and Fe oxides of Molisolls of Southeastern Buenos Aires province and the changes originated by NT, like stratification of P and OM [61]. Therefore, changes in concentration of Bray-P in depth



(a) (b)

(c)

FIGURE 4: Spring wheat grain yield affected by P placement method and P rate. Means followed by the same letter are not significantly different from each other based on the Duncan test (0.05). Phosphorus response is compared using 0, 25, 50, and 150 P rates (gray). Phosphorus placement method is only compared at 25 and 50 P rates (Tandil and Necochea). P-B: phosphorus broadcasted. P-L: phosphorus banded at sowing.

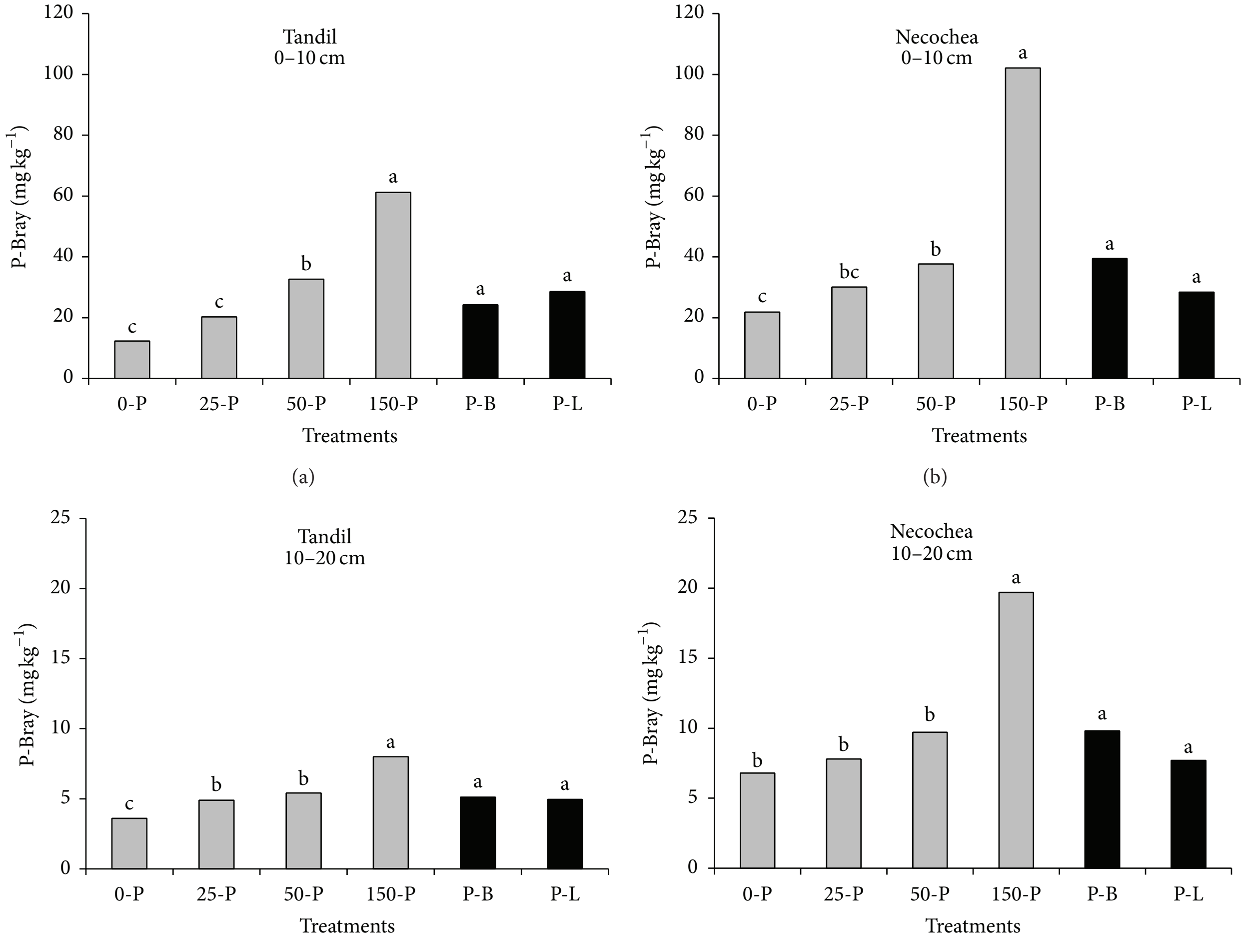
determined in this experiment could be the consequence of low P retention capacity of the soil surface layer under NT and the precipitation fell between broadcast P and soil sampled that is in concordance with a period to soil profile recharge water. These processes could have contributed to increase P availability for the wheat crop after tillering. Low P-fixing capacity in soils of this area were informed by Picone et al. [62].

3.6. Arbuscular Mycorrhizal Colonization. Highest AMC of wheat roots was found in the unfertilized treatments both at tillering and at flowering stages (Figure 6). Fertilization with 25 and 50 kg P ha^{-1} deep-banded decreased AMC for both sampling times and depths. However, the increase in the P rate did not produce similar marked depressions in AMC when the P was broadcast. Although available soil P was higher in the upper soil layer than in the lower (Figure 5), there were no differences in colonization between soil depths. In the upper soil 0–10 cm we found greater AMC at tillering for broadcast P at 25, 50, and 150 kg P ha^{-1} than in deep-banded P at 25 or 50 kg P ha^{-1} (Figure 6). Although differences were reduced at flowering and sometimes were not significant, threefold higher AMC in 25 and 50 kg ha^{-1} broadcasted treatments than in deep-banded ones were found. Because arbuscules had a similar behaviour to AMC with regard to fertilization treatments and soil depth (data are shown), we believe that fertilization and P placement affected arbuscules in the same way.

TABLE 5: Analysis of variance of P recovery (%) at Necochea affected by P rate and soil depth.

ANOVA	
Source of variation	$P > F$
P rate (R)	0.003
Soil depth (D)	0.012
$R \times D$	ns
Average treatments	
P rate (μg g soil^{-1})	% retention
60	33.2 a
120	28.5 b
Soil depth (cm)	
0–10	29.1 b
10–20	32.6 a

Means in the same column followed by the same letter are not significantly different from each other based on the Duncan test (0.05). ns: not significant.



(a) (b)

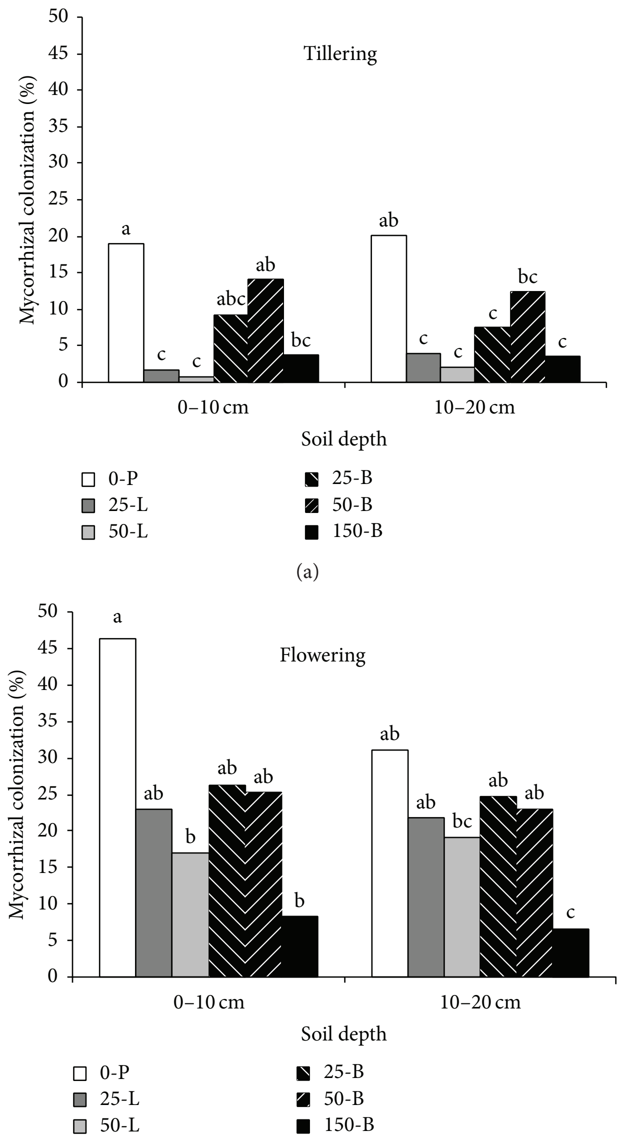
(c) (d)

Figure 5: Available soil P-Bray at tillering (0–10 and 10–20 cm) affected by placement method and P rate. Means followed by the same letter are not significantly different from each other based on the LSD test (0.05). Phosphorus response is compared using 25, 50, and 150 P rates (gray). Phosphorus placement method is only compared at 25 and 50 P rates. P-B: phosphorus broadcasted. P-L: phosphorus banded at sowing.

Covacevich et al. [37] showed that there was a negative relationship between AMC colonization of wheat roots (recovered both under field and glasshouse condition) and soil P availability at 0–20 cm of soil depth. At this experiment the AMC at the 0–20 cm depth of soil was also negatively correlated with available soil P ($r^2 = 0.85$, $P = 0.01$). The mycorrhizal colonization was related to current available soil P through a unique function AMC = 5.6 + 857.9/(Bray P)^2, both for fertilized (broadcasted and banded) and unfertilized treatments at the two phenological stages. The rate of decline in AMC was higher in the range of 6–13 mg kg^{-1} Bray-P, and decreased above this value. In general, at similar levels of soil P, values of AMC for broadcast P treatments were located above the trend line. This is another indication that broadcasted P decreased to a lesser extent the AMC in relation to line-banded applications. This could suggest more soil P uptake of root plants by banding but more mycorrhizal symbiosis uptake by broadcast P placement.

The extensive hyphal networks of AMF influence soil physicochemical properties and can directly or indirectly contribute to the release of soil P from inorganic complexes of low solubility [63]. In undisturbed soils, roots follow preformed channels, making close contact with the AMF-infected root system, resulting in enhanced AMC of the roots [21] and nutrient uptake. In Necochea, differences between P placement and AMC were found although these were not statistically significant ($P > 0.05$). Plots with broadcast P had the highest available soil P and were always associated with high mycorrhizal development, mainly at tillering. It is probable that this has contributed to a high P uptake by roots with the consequent increase in plant growth. This may also partially explain the absence of differences in yield and plant P content between P placement treatments in latter stages of growth. AMC was recorded at only one-site, so that our results constitute only the beginning to understand possible contribution of mycorrhiza to accessibility of wheat roots to soil P, especially under broadcast P applications.

Excess chemical fertilizer in soils is a major environmental concern and accumulation of P applied in excess can increase the risk of P movement to surface and groundwater [64]. Therefore, it is important that P management balances the goal of providing sufficient P to the crop to



(a)

(b)

Figure 6: Indigenous mycorrhizal colonization of wheat roots at the Necochea site as affected by P fertilization and soil depth. Means followed by the same letter are not significantly different from each other based on the LSD test (0.05). P-B; phosphorus broadcasted. P-L: phosphorus banded at sowing.

optimize crop yield with the goal of avoiding excess P and environmental risk. Grant [65] pointed out that side banding of P could be better for crops (mainly in conditions with cold soil temperatures because of slower P diffusion rate). Moreover, she pointed out that in some cases mycorrhizal colonization could be detrimental to the plant (higher cost in photosynthesis than in P uptake benefit) because wheat seems to not be highly dependent on the mycorrhizal association to access soil P as a result of its extensive root development. However, for these conditions, our results are in disagreement with those reported by Grant [65]. It is probable that, without mycorrhiza formation, wheat plants in broadcast P treatments would be at a larger distance from the root system, which could limit the P availability to the root, in relation to P dep-banded adjacent to the seed. Thus, it is probable that high mycorrhiza formation, which was less depressed by the soil P in broadcasted treatments, aimed to connect the soil P with the roots allowing adequate access to nutrient. These results allow speculation that, under NT, the AMF could have functioned as "facilitators" of available soil P for wheat plants when P was broadcasted. Moreover banding P increased disturbance and could produce damage to the hyphal network reduce AMF growth and root colonization due to death or reduced infectivity of the hyphal fragments compared with intact networks and by detaching them from the host plants [21, 22]. However, the present study only provides the first steps in this direction and cannot confirm the possible mechanisms involved. Therefore, more comprehensive studies are needed to understand the contribution mycorrhiza to wheat associated with P placement.

4. Conclusions

These results indicate that in soils under NT with low available P, high OM and water content in the surface horizon, P placement method does not affect wheat grain yield, plant P uptake and recovery efficiency, independent of P rate. The lack of differences observed could be explained by a low P retention capacity in the soils where the research was conducted and maybe by a higher indigenous mycorrhiza formation in roots with applications of P broadcasted compared with P deep-banded.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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