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***Phosphorus fertilization, soil phosphorus availability
and grain yield of durum wheat (*Triticum durum* Desf.)
and maize (*Zea mays* L.) under no-tillage and reduced
tillage conditions***

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General Introduction

1. Preface

The depletion rate of reserves for phosphorus fertilizer production are expected to increase, as the world population will grow by another 3 billion people over the next 40 years (United Nations, 2007). The agricultural sector results in the depletion of approximately 19 Mt y⁻¹ of phosphorus from phosphate rock for fertilizer production (Heffer and Prud'homme, 2008) and the easily accessible phosphate rock reserves are likely to be depleted within 50–150 years. The critical point of phosphorus extraction is predicted to occur by 2035 after which the demand could not be satisfied (Cordell et al., 2009). On the other hand, phosphate rock reserves are decreasing in grade (% P₂O₅ content) and accessibility, requiring additional energy inputs and costs.

In order to contrast this scenario, thus, reduce the global demand for phosphorus and postpone the depletion of fossil reserves, improve the phosphorus use efficiency in agriculture and in particular the fertilizers use efficiency, can be some of the most important ways (Cordell et al., 2009).

As suggested by Schröder et al. (2011), phosphorus use efficiency can be improved optimizing the use of agricultural land, maintaining soil quality, preventing erosion (Indoria et al. 2017) (that is also one of the key factors of conservation agriculture) and improving and adjusting fertilizer recommendations, placement, input and output.

The awareness of the described issue and the necessity to assure high yield in agriculture, have given rise to new approaches to improving phosphorus use efficiency in terms of fertilizers (Herrera et al. 2016). In this context, a new type of phosphorus fertilizers based on organomineral phosphorus complexation were developed in order to improve the stability of phosphorus in soil, enhancing its use efficiency and uptake by crops (Baigorri et al. 2013).

Recent studies (e.g. Baigorri et al. 2013; Urrutia et al. 2014; Herrera et al. 2016) proved that the complexation of humic substances with the phosphate fraction of fertilizer complexes are able to decrease phosphate fixation in the soil and increase phosphate uptake and consequent plant growth. These new phosphorus fertilizers proved also to be efficient to improve plant growth parameters with respect to conventional fertilizers.

Being a relatively new product in the market, there is still lack of literature, especially regarding cereal crops in the European area. For this reason, the aim of this PhD thesis is to evaluate the improvement in agronomic and phosphorus use efficiency of humic-complexed fertilizer as showed in the analyzed literature, comparing the humic-complexed phosphorus fertilizer with a standard formulation (triple superphosphate) on durum wheat and maize (two of the principal annual crops

cultivated in the world) studying the differences in soil phosphorus availability, plant growth and grain yield.

2. Structure of the thesis

The body of the thesis includes three manuscripts based on researches carried out during the period of PhD:

1. The first chapter is a *Systematic Review* entitled: **“Phosphorus fertilization and soil phosphorus availability in no-tillage systems: a systematic review with a focus on wheat crops”**. The review aims to enlarge our understanding of phosphorus behavior in no-tillage systems, to maximize its use efficiency and diminish its environmental losses, with a focus on wheat crops. A total of 58 papers were analyzed according to a systematic review approach, to highlight spatial and temporal trends, climate zones, soil types, fertilization typologies and doses, trial durations. The aims of these studies and their main findings were also analysed. These data show: (i) an increasing trend of publications over recent years with a non-homogenous distribution of case studies; (ii) studies on soil phosphorus availability in no-tillage wheat systems can produce uncertainties and contrasting data, which are also strongly context dependent; (iii) long-term studies are necessary to fully characterize phosphorus dynamics in no-tillage wheat systems; (iv) site-specific calibration of phosphorus fertilization is necessary to avoid over- or under-fertilization; and (iv) as mineral phosphorus sources are limited and being depleted, there is the need for future studies to be directed toward alternative forms of organic fertilization.
2. The second chapter is a full research manuscript entitled: **“Phosphorus fertilization, soil phosphorus availability and grain yield of durum wheat (*Triticum durum* Desf.) under no-tillage conditions”**. Researches regarding phosphorus availability in no-tillage systems, plant growth and grain yield through the application of new product as humic-complexed phosphate fertilizers is still restricted in literature especially concerning wheat cultivated under Mediterranean climate conditions. In this perspective, a two year experiment was carried out to investigate the effects of two different phosphorus fertilizers (humic-complexed phosphate and triple superphosphate) under Mediterranean and no-tillage conditions on i) soil phosphorus availability, ii) dry matter and dry matter translocation iii) phosphorus use efficiency and iv) grain yield and grain components of durum wheat (*Triticum durum* Desf.).

3. The third chapter is a full research manuscript entitled: **“Phosphorus fertilization, soil phosphorus availability and grain yield of maize (*Zea Mays L.*) under reduced tillage conditions”**. This chapter is focused on a two year experiment where two type of phosphorus fertilizers (humic-complexed phosphate and triple superphosphate) are compared on maize system in reduced tillage conditions. The objectives of this research are i) evaluate the soil phosphorus availability conferred by different fertilizer rates under two different type of fertilizers, humic-complexed phosphorus and triple superphosphate, ii) study the plants architecture (leaf area index, plant height and dry matter) and iii) evaluate the differences in grain yield to identify the best rate and type of phosphorus fertilizer, to ensure high production and a better sustainability.

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Chapter I: Phosphorus fertilization and soil phosphorus availability in no-tillage systems: a systematic review with a focus on wheat crops

1. Introduction

Phosphorus (P) is an important element for plant nutrition that cycles through the plant–soil system as a component of vital biochemical molecules (e.g., nucleic acids, phospholipids) and it is among the most limiting factor for plant growth and productivity in agricultural soils (Holford, 1997). Scarce availability of P is due to its soluble ionic form that generally reacts with calcium, iron and aluminium, depending on soil pH (Hinsinger, 2001; Piegholdt et al., 2013; Sharpley et al., 2003).

Although P fertilization in agricultural soils has contributed to increased crop yields over the past century, P fertilizers are currently highly overused in crop production. This is especially in developed regions where the accumulation of P in the soil and eutrophication of natural aquifers has been shown to be a threat to the environment in many studies (Tilman et al., 2002; Redel et al., 2007; Ma et al., 2011; Piegholdt et al., 2013; Wu et al., 2016; Wang et al., 2016). Furthermore, human activities have significantly depleted the natural reserves of P, and it is estimated that they will be exhausted within 50 to 400 years, which will lead to increased costs of P fertilizers (Smil, 2000; Cordell et al., 2009; Sattari et al., 2012; Scholz and Wellmer, 2013; Reijnders, 2014).

It is clear that a more integrated and effective approach to P management in agricultural systems is highly needed (Smil, 2000; Redel et al., 2007; Cordell et al., 2009; Gilbert, 2009; Vaccari, 2009; Fontoura et al., 2010; Brennan et al., 2013). In particular, there is the need to highlight how the rate of available P can change due to new conservation techniques (e.g., minimum or no-tillage [NT], cover crops) or the application of new P-fertilizer formulations promoted for low-impact agro-ecosystems.

The specific use of NT is largely recognized as providing minimal soil disturbance while favoring accrual of organic matter. It is also a common practice in so-called ‘conservation agriculture’, which is based on three particular general crop-management principles: (i) reduced disturbance of the soil; (ii) optimized crop rotation; and (iii) cover crops to permanently cover and protect the soil (FAO, 2011). The worldwide expansion of NT started in the mid-to-late 1990s, and it was facilitated by the use of herbicides and improved NT technologies (Derpsch et al., 2010). Nowadays, according to Kassam et al. (2014), NT has been adopted for almost 11% (155 Mha) of the global arable land area, especially because it requires reduced energy and machinery inputs compared to conventional tillage (Triplett and Dick, 2008; Zhang et al., 2017). NT is also increasingly recognized as providing environmental benefits through reduction of soil erosion (Scopel et al., 2005;

Verhulst et al., 2010), improvement of soil fertility (Madari et al., 2005), and enhancement of soil carbon sequestration (González-Sánchez et al., 2012).

Conservation agriculture principles and NT are largely applied to cereal systems. As such, wheat (*Triticum aestivum* L.) represents the third most commonly grown crop in the world, with over 200 Mha cropped annually (Rajaram and Braun, 2008), and it provides more than 25% of the world total cereal output (Manske et al., 2001). The influence of NT on wheat yield in the USA has been studied in depth (De Vita, 2007), as also for Europe (Mazzoncini et al., 2008; Soane et al., 2012; Grigoras et al., 2013), Australia (Armstrong et al., 2003; Fabrizzi et al., 2005; Llewellyn et al., 2012) and Asia (Li et al., 2007; Shao et al., 2016). It has been shown that wheat cropping systems can benefit from NT in terms of many different aspects, such as grain yield (De Vita et al., 2007), mycorrhizal colonization (Brito et al., 2006), use efficiency of water (Bonfil et al., 1999), and reduction of herbicide use (Bräutigam and Tebrügge, 1997). However, contrasting data have emerged in the literature regarding the relationship between NT and grain yield (e.g., Hernanz et al., 2002; Mazzoncini et al., 2008), and little is known about the availability of nutrients, and especially of P, in NT systems.

Therefore, enlarge our understanding of P behavior in NT soil systems practices, it is crucial to maximize the use efficiency of P and to enhance wheat crop production while diminishing P losses to the environment. In the light of these needs, the adoption of a methodological research approach that helps in the definition of quality studies might be useful. A systematic review will allow the integration of information from a wide set of studies that might present conflicting and/or overlapping data, so as to identify the aspects that lack evidence, and thus to indicate the directions needed for future research (Linde and Willich, 2003).

In line with this approach, this systematic review aims to identify knowledge gaps by addressing the following questions: (i) How has the relevant scientific literature evolved over the recent decades? (ii) Where and to what extent has NT influenced soil P availability? (iii) At what soil depth does P tend to be located and more available to plants? and (iv) Is grain yield influenced by soil P under NT systems? To answer to these questions, this study reviewed 58 relevant peer-reviewed research papers on soil P availability in NT systems, with a specific focus on wheat.

2. Materials and methods

In December 2016, the following search terms were entered in both the Web of Knowledge and Scopus databases: “*no tillage*”; “*phosphorus*” OR “*P*”; “*fertilizer*” OR “*fertilizing*”; “*wheat*” OR “*triticum*”; “*soil*”. The articles retrieved were subjected to a selection procedure to identify the

studies that fit with the aim of the present review. Articles that partially fit with the research topics (e.g., papers dealing with NT, but not with P fertilization or wheat crops) were not eligible. The study was not limited to any specific time period (e.g., 30 years), however, it can be noted that ‘gray literature’ might have been excluded. Nevertheless, the papers included in this review provide clear views of the topic issues.

The eligible papers were analyzed according to a systematic review approach (Pickering et al., 2014) that differed from classic meta-analysis, as it did not analyze published data but identified geographic, theoretical, and methodological gaps by analyzing literature trends. Systematic reviews can provide quantitative summaries of an analyzed field topic and report structured data that can be used as the initial step for further analysis (e.g., meta-analysis) (Pickering and Byrne, 2014). The selected papers were analyzed to highlight their findings on P availability under conservative NT practices within wheat cropping systems, and for the characteristics of the publications (i.e., year of publication, location, Köppen-Geiger climate type, soil type (according to Food and Agriculture Organisation of the United Nations/ US Department of Agriculture), fertilization type, length of experimental period, research objectives, main findings and their spatiotemporal trends.

The data are reorganized graphically and are summarized in Tables A1, A2 (see Appendix), to efficiently gather the main findings from the available scientific literature. These are finally compared in the Discussion section. A similar analysis approach for data in systematic reviews was adopted by Rupprecht et al. (2015) and Rupprecht and Byrne (2014).

3. Results

3.1. Eligible studies: papers and journals

The search terms returned a total of 399 papers, of which 58 were selected as in line with the aims of this review (i.e., papers dealing simultaneously with NT, P fertilization, wheat). The 58 papers were divided in 56 original researcher papers, 1 short communication, and 1 review paper. They were distributed across 27 different journals, with the journals that published most of these research papers as the following: *Communications in Soil Science and Plant Analysis*, *Agronomy Journal*, *Canadian Journal of Soil Science*, *Soil and Tillage Research*, and *Revista Brasileira de Ciência do Solo*. The variety of journals and authors underlines the shared interest in this research topic. A summary of the findings that includes the climate zone, soil type, length of experimental period, aims of the studies, and relevant main findings is given in the Appendix (Table A1). The next paragraph details the data that emerged from this analysis.

3.2. Trends and patterns in the literature

The earliest study among those selected was conducted by *Tracy et al.* (1990). During the 1990s and up to 2005, the number of publications was relatively constant (1990s: 0.9 year⁻¹, on average). This included a slight increase in the number of papers that were focused on the specific topic in 2001. From 2006 to 2016, the number of papers rose (3.9 papers years⁻¹, on average), with the peak in 2013 (7 papers) (Figure 1).

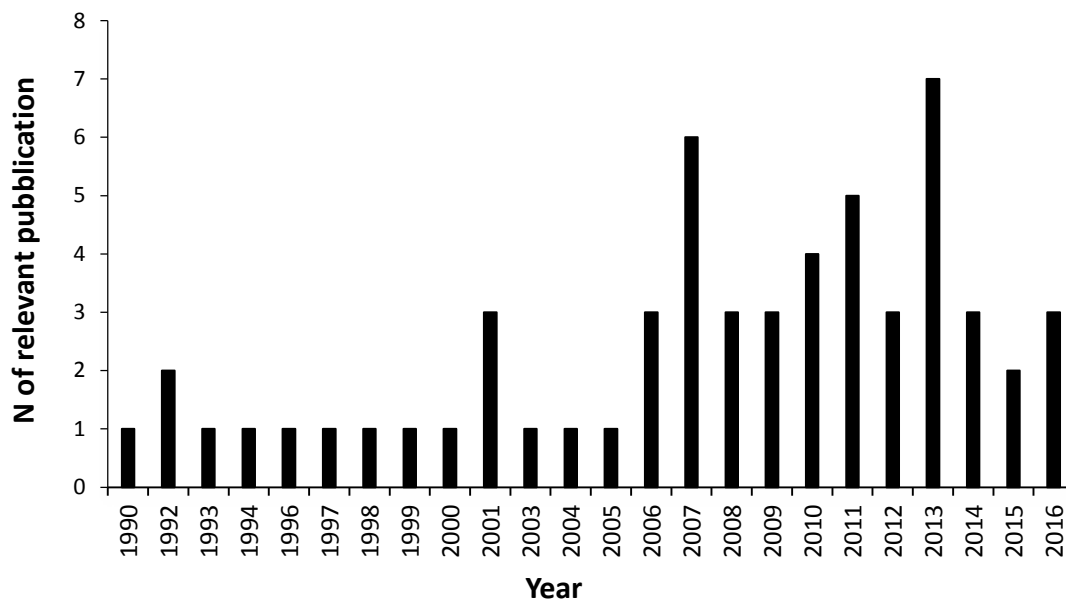


Figure 1: Publication history of the selected papers.

3.3. Spatial patterns

The selected papers showed heterogeneous geographic distributions, with most studies carried out in only a few countries (Figure 2). North America was the most represented area, with 25 papers (USA, 16; Canada, 8). Studies in South America were mostly conducted in Brazil (9), followed by Argentina (4), Chile (1) and Paraguay (1). A few papers reported on studies in Asia (India, 4; Pakistan, 1; China, 1), while eight studies were conducted in Australia. Europe was maybe surprisingly poorly represented, with one case study across three nations (Germany, Lithuania, Romania). Finally, two studies were conducted in Africa (Morocco, South Africa).

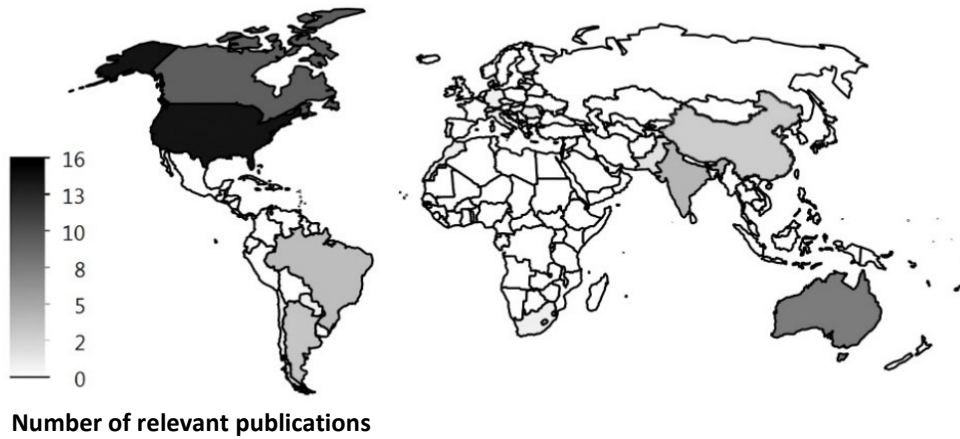


Figure 2: Geographic distribution of the selected papers.

3.4. Climate zone distribution

The climate distribution of the papers was defined according to the Köppen-Geiger classification, thus grouping the climate conditions of the study areas according to the letters: A, tropical; B, dry; C, temperate; and D, continental (Figure 3). The data showed that most of the studies were conducted under temperate climatic conditions (20), followed by dry (18), continental (18) and tropical (3) climates.

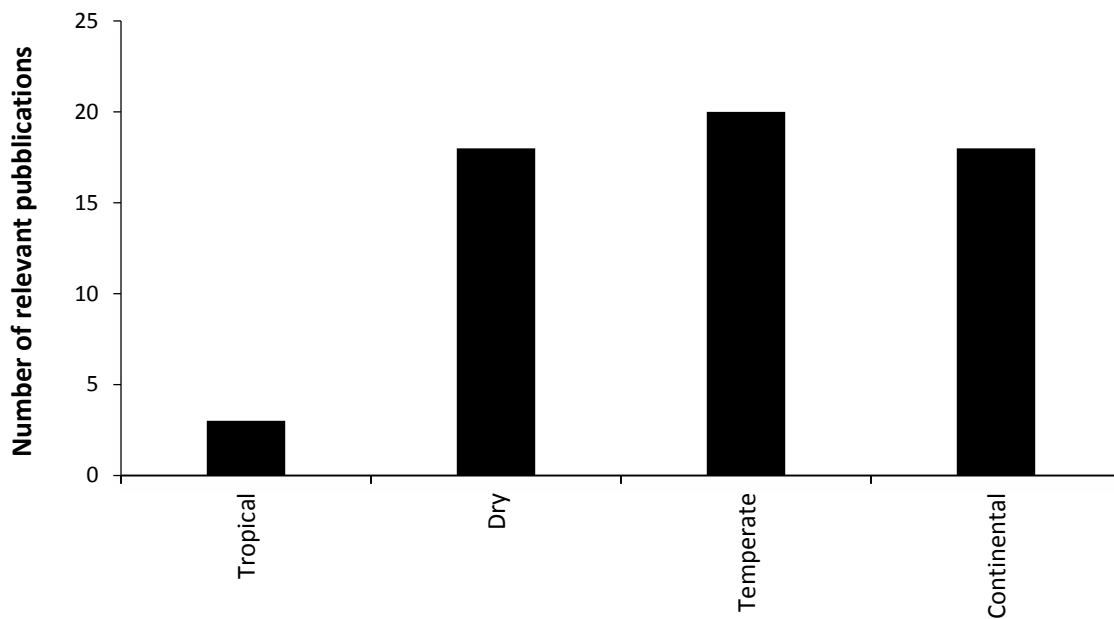


Figure 3: Distribution of selected papers according to Köppen-Geiger climate groups.

3.5. Aims of the research papers

Eight main research aims were identified, with the purpose of obtaining better understanding of the issues concerning the topic of this review: crop yield, fertilization, tillage, soil physico-chemical qualities, soil P availability, P uptake, P placement, and others. Table 1 summarizes and defines these different aims in more detail. The higher number of aims (n = 118) compared to the number of selected papers (n = 58) is due to the definition of multiple aims within each article.

Table 1: Aims and description of the relevant studies.

Study aims	Description	No. of papers
Crop yield	Grain production related to P fertilization	27
Tillage	Different tillage systems (NT, conventional, reduced)	22
Fertilization	Different doses and/or types of fertilizers	19
Soil P availability	Soil P availability (through soil analysis)	17
Soil physico-chemical qualities	Changes in physical or chemical characteristic of soil in relation to tillage and P fertilizers	8
P uptake	P uptake at maturity or during growth stages in grain, straw, or above-ground plant. P recovery efficiency and P use efficiency	9
P placement	The best way to distribute P as a fertilizer	10
Other	Others aims (e.g., indigenous mycorrhizal colonization; Covacevich et al., 2008)	6

Different types of treatments were applied within the experimental trials. The most used were inorganic fertilizers (34), while organic fertilizers were used in only eight trials (i.e., mulch, compost, crop residues, manure). However, a large number of the papers did not specify the type of fertilizer used (23). All of the types of fertilizers are showed in Figure 4.

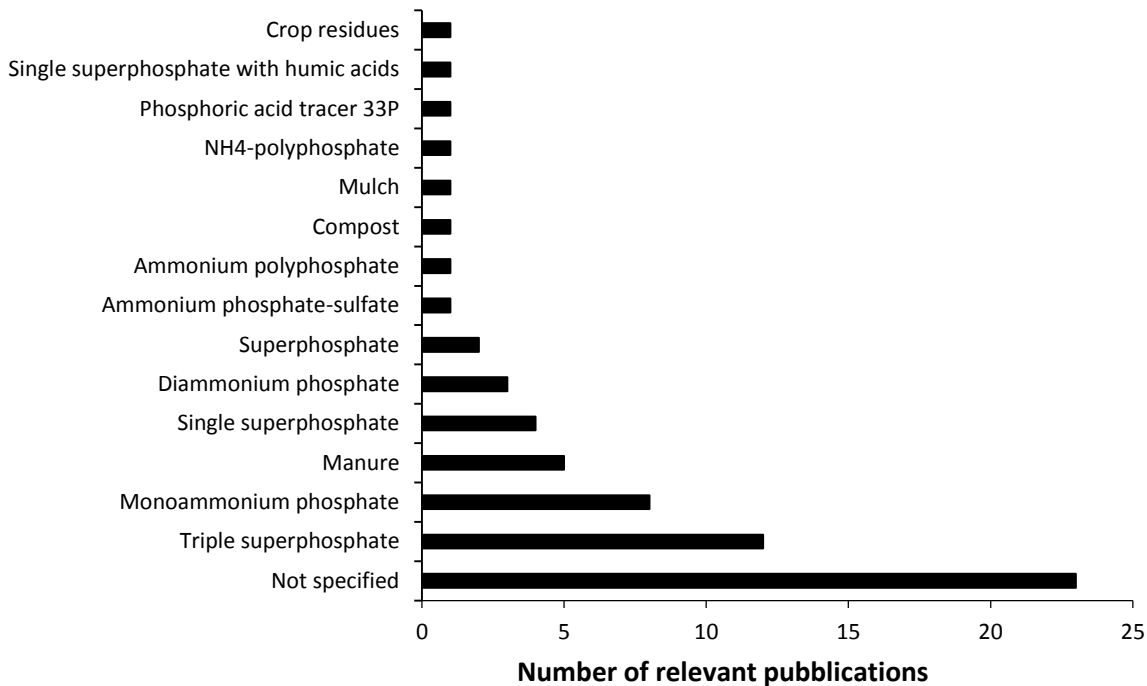


Figure 4: Type of fertilizers used in the experimentation trials in the selected papers.

3.6. Durations of the experimental trials

Most of the studies lasted from 1 to 5 years (35), although some long-term studies emerged from the analysis. Indeed, five papers reported experimental trials of 20-30 years duration (i.e., Campbell et al., 2011; Tiecher et al., 2012a, b; Brennan et al., 2013; Karamanos et al., 2013), and two papers included studies of longer than 30 years (i.e., Feiza et al., 2010; Loke et al., 2013).

4. Discussion

4.1. Gaps of knowledge in the literature

The analyzed papers highlighted gaps of knowledge for three main aspects: (i) geographic distribution of the studies, (ii) study duration; and (iii) study of organic P in the soil.

Five countries (i.e., USA, Canada, Brazil, Australia, Argentina) dominated the study of P in NT systems with wheat crops. At the same time, very little has been reported on this topic from Europe, Africa and the oriental countries. In a broad sense, studies are still needed to demonstrate the benefits of NT on soil fertility and crop yields under local conditions (Loke et al., 2013). The filling of this knowledge gap appears to be of major importance, because the data from these studies tend to be site-specific and might vary across different conditions of climate and soil (e.g., texture, chemical

characteristics, pH). For these reasons, future studies of the wheat response to P dynamics in NT systems need to be performed in relation to further, and different, geographic contexts.

The study duration of field trials emerged as an additional knowledge gap. This review has highlighted that most studies were carried out over a period of between 1 and 5 years, with only a few carried out for longer periods. However, studies on the fate of residual P fertilizer and on the effects of P-fertilizer application on soil P pools under NT conditions on wheat crops systems need to cover the long term (Mao et al., 2015). In general, the research topics analyzed in the present review would demand longer periods, to better understand the different dynamics and to obtain sound long-term data.

The third aspect of the lack of knowledge highlighted by the present review relates to the soil organic P and organic P fertilization. From the articles analyzed, it emerged that the most used form of fertilizer was inorganic P, and a considerable amount of information about the dynamics of inorganic P in the soil is available. On the contrary, as showed in Figure 4, only nine papers applied and investigated organic fertilizers, probably because of the difficulties with the incorporation of P into the soil. It is known that generally organic P declines rapidly under continuous cropping systems without application of P fertilizers, simultaneously with the decrease in total organic carbon and total P (Solomon and Lehmann, 2000; Conte et al., 2002; Solomon et al., 2002). However, little is known concerning the factors that contribute to increases in soil organic P (Tiecher et al., 2012a). For these reasons, future studies need to address organic P dynamics in NT systems that especially take in consideration the current depletion of phosphate rock reserves, which in 2035 will not be able to satisfy demand (Cordell et al., 2009).

4.2. Soil P availability and soil P concentration

Many studies indicated that P levels in the soil were affected by climate, soil texture, amount and type of fertilizer used, tillage system, and crops, which might also affect the distribution of P into different pools (Shafqat and Pierzynski, 2010; Piegholdt et al., 2013). P availability might be related to tillage practices, as many of these studies reported higher P under NT, compared to conventional tillage systems (Selles et al., 1999; Mrabet et al., 2001; Thomas et al., 2007; Schlindwein and Gianello, 2008; Tiecher et al., 2012a; Loke et al., 2013).

The conversion from tilled to NT systems was also demonstrated to highlight positive variations in soil P availability (Selles et al., 1999). In NT systems, stubble mulch and crop residues left in the field have an important role in terms of P availability (Loke et al., 2013). These increase the rate of release of P through decomposition and the potential for plant roots to access P (Ball-Coelho et al., 1993; Noack et al., 2014) and to increase the soil organic P (Tiecher et al., 2012a).

Moreover, NT systems can increase the organic matter content in the upper soil layer, and this can stimulate soil acidification in the seed zone (in calcareous soils), which raises the P availability for wheat crops (Mrabet et al., 2001). On the other hand, some studies showed no significant differences in P availability between NT and conventional tillage systems (Singer et al., 2004; Schwab et al., 2006; Piegholdt et al., 2013), or less P availability under NT with respect to other tillage systems (Iqbal et al., 2011). Lastly here, Daroub et al. (2001) showed that the adoption of NT systems in rotation over various years did not increase the organic P significantly.

An important issue that was widely studied here and is reported in this review is the P concentrations in the soil layers under NT cropping systems. In the literature, it has been established that under most soil conditions and compared to the other elements, P is the least mobile and available element for plants (Hinsinger, 2001). The papers analyzed clearly show that P concentrations were generally studied in two soil layers, as 0-10 cm and 10-20 cm. According to Schindwein and Gianello (2008), the critical P concentrations are greater in soils under NT in both the 0-10 cm and 0-20 cm soil layers. However, the majority of the studies reported that the effects of NT on P availability were confined to the topsoil, with P strongly stratified in the first (0-5; 0-10) cm layers (Mrabet et al., 2001; Thomas et al., 2007; Covacevich et al., 2008; Vu et al., 2009; Calegari et al., 2013; Kushwah et al., 2016). The reason of this might be related to the low mobility of P. From what was shown in the other studies, it appears that for the conventional system of tillage, and in particular with ploughing, P can be driven to greater depths. In contrast, in NT systems, P movement is influenced mainly by soil texture and climate conditions.

4.3. Wheat production

Contrasting data came out in terms of wheat production. Although a considerable number of studies reported that in NT systems P fertilization increased wheat grain yield (Lutcher et al., 2010; Coventry et al., 2011; Herrera et al., 2016), many other studies did not find any effects of tillage system on wheat grain yield (Rasmussen and Douglas, 1992; Rehm et al., 2003; Singer et al., 2004; Schwab et al., 2006; Grigoras et al., 2013; Karamanos et al., 2013). Although the application of P fertilizers might be the most direct and efficient approach to increase wheat production in many cases (Zhu et al., 2012), application of P fertilizers to soils with high available P contents proved not to be necessary (Fontoura et al., 2010; Brennan et al., 2013). According to Lafond et al. (2001), there was a positive yield response with soil P levels of 24 kg ha⁻¹, but no response for soil P levels greater than 34 kg ha⁻¹. Finally, as reported by Brennan et al. (2013), the application of P fertilizers when not required might not affect grain yield, but might enhance the P status of the soil and increase the risk of run-off and water contamination, with subsequent eutrophication.

5. Conclusions

This systematic review analyzed 58 peer-reviewed scientific articles on P fertilization in NT systems on wheat crops, with the aim to identify major literature gaps and provide an overview of the state of the art. The increasing trend of publications over the last years shows that the topics analyzed are currently under investigation. However, the nonhomogenous distribution of case studies around the world suggests that NT and P relations on wheat crops have been more deeply investigated in only a few countries (i.e., USA, Canada, Brazil, Australia, Argentina), and not particularly in others. A very relevant gap of knowledge is thus represented by the limited number of studies in countries where wheat-based systems are dominant (e.g., Mediterranean countries, India).

Although most of the studies concluded that the higher soil P concentrations are in the top soil layers (0-10 cm), soil P availability in NT wheat systems still represents a major challenge for researchers, as contrasting data and uncertainties emerged in the present review. If, in general, wheat grain yield is affected by soil-available P, this review has highlighted that NT produces different and contrasting data depending on the site-specificity of the studies.

In conclusion, the present review shows that: (i) there is the need to expand the study areas in which P dynamics are investigated in NT wheat systems; (ii) long-term studies are necessary to fully characterize P dynamics in NT wheat systems; (iii) site-specific calibration of P fertilization must be implemented, to avoid over- or under-fertilization; and (iv) as mineral P sources are limited and under depletion, future studies should be directed toward the use of organic fertilization.

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7. Appendix

Table A1. Details of the 58 research articles analyzed in the present review.

First author	Year	Place of study	Climate (Köppen)	Soil type (FAO ^a ; USDA ^b)	Fertilization type	Fertilization kg ha ⁻¹ P (P ₂ O ₅) ^c	Trial duration (years)
Armstrong, R.D.	2015	North-western Victoria, Australia	Cold semi-arid	Vertosol, Calcarosol ^a	Phosphoric acid tracer ³³ P	15	16
Bailey, K.L.	1998	Indian Head; Rapid City, Canada	Humid continental	Black Chernozem ^a	Monoammonium phosphate	0-8-16	2
Barbieri, P.A.	2014	Southeastern Buenos Aires, Argentina	Mesothermal-humid–subhumid	Typic Argiudolls ^b	Diammonium phosphate	0-25-50-150	1
Bhaduri, D.	2014	New Delhi, India	Semi-arid; sub-tropical	Typic Haplustepts ^b	Single superphosphate	26.2	7
Bolland, M.D.A.	2006	South-western Australia	Hot semi-arid	NS	Triple superphosphate	0-5-10-20-40	3
Brennan, R.F.	2013	Australia	Cold semi-arid	Typic Natrixeralf	Triple superphosphate	From 5 to 11	21
Brye, K.R.	2007	Mississippi River delta region, Arkansas, USA	Humid subtropical	Typic Glossaqualf ^b	NS	0	2
Buchanan, M.	1994	Raleigh, North Carolina, USA	Humid subtropical	Typic Kanhapludults ^b	NS	NS	2
Calegari, A.	2013	Paraná State, Brazil	Humid subtropical	Rhodic Hapludox ^b	NS	568	19
Campbell, C.A.	2011	Saskatchewan, Canada	Subhumid continental	Black Chernozem ^a	Monoammonium phosphate	11	20
Chen, W.	2009	Australia	NS	NS	NS	NS	NS
Covacevich, F.	2008	Necochea, Argentina	Oceanic	Hapludol ^b	Triple superphosphate	0-25-50-150	5
Coventry, D.R.	2011	Haryana, India	Hot desert	NS	Diammonium phosphate	26	2
Cubilla, M.M.	2007	South-east Paraguay	Humid subtropical	Ultisol, Oxisol ^a	NS	0-50-100-200-400	2
Daroub, S.H.	2001	Hickory Corners, Michigan, USA	Warm humid continental	Hapludalf ^b	NS	0	11
Divito, G.A.	2010	Southeastern Buenos Aires, Argentina	Mesothermal-humid–subhumid	Typic Argiudolls ^b	Triple superphosphate	70	2
Essington, M.E.	2000	Milan, Tennessee, USA	Humid subtropical	NS	NS	0-20-60	9; 10

Feiza, V.	2010	Kėdainiai district, Lithuania	Warm humid continental	Cambisol ^a	NS	NS	53
Fontoura, S.M.V.	2011	Paraná State, Brazil	Humid subtropical	Oxisol ^a	Triple superphosphate	0-40-80-160	6
Grigoras, M.A.	2013	Cluj County, Romania	Warm humid continental	NS	NS	40; 75	1
Halvorson, A.D.	1992	Colorado, USA	Cold semi-arid	Aridic Argiustolls- Calcicustoll ^b	Single superphosphate; Manure	From 0 to 134	2
Herrera, W.F.B.	2016	Tibagi, Brazil	Humid subtropical	Typic Hapludox ^b	Single superphosphate with humic acids	96	4
Iqbal, M.	2011	Faisalabad, Pakistan	Semi-arid	Calcic Argids ^b	NS; Mulch	115; [0-2-4-6 (P in mulch)]	2
Jackson, G.D.	1993	Montana, USA	Cold semi-arid; Humid continental	Tamaneen; Scobey; Phillips; Kevin; Marias ^b	Monoammonium phosphate	From 0 to 25	4
Jackson, G.D.	1997	Montana USA	Cold semi-arid; Humid continental	Tamaneen; Scobey; Phillips; Kevin; Marias ^b	Monoammonium phosphate; Triple superphosphate	From 0 to 29	9
Karamanos, R.E.	2013	Alberta, Canada	Warm humid continental	Gray Luvisol, Chernozemic ^a	NS	17; 20	30
Kelley, K.W.	2007	Kansas, USA	Humid subtropical	Mollic Albaqualf ^b	NS	NS	5
Kushwah, S.S.	2016	India	Sub-humid tropical	Typic Hapluster ^b	Single superphosphate; Manure	30; 30	5
Lafond, G.P.	2001	Southern Saskatchewan, Manitoba, Canada	Warm humid continental	Black Chernozem ^a	Monoammonium phosphate	0-9-17	2
Liu, C.A.	2013	Yuzhong County, Gansu Province, China	Medium-temperate semi-arid	Calcic Kastanozems ^b	Sheep manure; Superphosphate	15,12; 15,7	7
Loke, P.F.	2013	Bethlehem, South Africa	Semi-arid	Plinthosol ^a	Single superphosphate	12.5	32
Lutcher, L.K.	2010	Adams County; Washington State; Morrow County, Oregon, USA	Warm-summer mediterranean	Calcic Haploxeroll ^b	Triple superphosphate	0-5-15	3
Malhi, S.S.	2008	Saskatchewan, Canada	Subhumid continental	Black Chernozem ^a	Monoammonium phosphate	7;10	3
Mooleki, S.P.	2010	Saskatchewan, Canada	Subhumid continental	Black Chernozem ^a	Monoammonium phosphate	7; 10	3
Mrabet, R.	2001	Sidi el Aydi, Marocco	Semi-arid	Xerosol ^a	Triple superphosphate	50	11
Noack, S.R.	2014	Southern Australia	Glasshouse experiment	Calcarosol ^a	NS; Crop residues	NS	1

Parsons, K.J.	2007	Canada	Humid continental	NS	NS; Liquid dairy manure	NS 14;22;29	2
Piegholdt, C.	2013	East and south Germany	Warm humid continental	Phaeozems and Luvisols ^a	Triple superphosphate; diammonium phosphate	4-7	12
Pradhan, P.R.	2011	New Delhi, India	Hot semi-arid	Typic Haplustept ^b	NS	60	3
Rasmussen, P.E.	1996	Pendleton, Oregon, USA	Semi-arid	Typic Haploxerol ^b	Ammonium phosphate-sulfate	12-24	1
Rasmussen, P.E.	1992	Pendleton, Oregon, USA	Semi-arid	Typic Haploxeroll ^b	NH4-polyphosphate	11	6
Redel, Y.D.	2007	Temuco, Southern Chile	Oceanic	Ultisol ^a	NS	200	3
Rehm, G.W.	2003	Crookston, Minnesota, USA	Warm humid continental	Aeric Calciaquoll ^b	NS	22	3
Schlindwein, J.A.	2008	Rio Grande do Sul, Brazil	Humid subtropical	NS	NS	0-62-123-246-492	2
Schwab, G.J.	2006	Bourbon County, Kansas, USA	Humid continental	Mollic Albaqualfs, Typic Argiudolls ^b	Ammonium polyphosphate	20	3
Selles, F.	1999	Southern Saskatchewan, Canada	Subhumid continental	Othric Brown Chernozemic ^a	Monoammonium phosphate	8.9	12
Singer, J.W.	2004	Boone, Iowa, USA	Hot humid continental	Typic Endoaquolls, Typic Hapludolls ^b	Compost	373; 389; 138	3
Singh, D.K.	2005	Mulga View; Roma, Australia	Semi-arid	Kandosol, Vertosol ^a	Triple superphosphate	10-40	1
Steiner, F.	2012	Paraná State, Brazil	Humid subtropical	Rhodic Hapludox ^b	NS; Manure	10; 10,2	2
Thomas, G.A.	2007	Southern Queensland, Australia	Humid subtropical	Abruptic luvisol ^b	Triple superphosphate	20	9
Tiecher, T. (a)	2012	Southwestern Paraná State, Brazil	Subhumid tropical	Oxisol ^a	NS	659	23
Tiecher, T. (b)	2012	Paraná State, Brazil	Humid subtropical	Rhodic Hapludox ^b	NS	1510 ^c	23
Tracy, P.W.	1990	Sidney, Nebraska, USA	Cold semi-arid	Pachic Haplustoll ^b	NS	NS	1
Vieira, R.C.B.	2016	Paraná State, Brazil	Humid subtropical	Oxisols ^a	NS	0-80-160-320-640 ^c	10
Vieira, R.C.B.	2015	State of Paraná, Brazil	Humid subtropical	Oxisols ^a	Triple superphosphate	0-80-160-320-640	5
Vu, D.T.	2009	Victoria, Australia	Cold semi-arid	Calcarosol ^a	Superphosphate	12	Different trials: >20

Yuan, Y.	2009	Leflore County; Sunflower County, Mississippi, USA	Humid subtropical	Typic Endoaqualf, Vertic Endoaquept, Chromic Dystraquer ^b	NS	21,9; 29,1; 112	4
Zamuner, E.C.	2006	Southeast Buenos Aires, Argentina	Mesothermal-humid– subhumid	Typic Argiudoll ^b	NS	50-110	1

NS, not specified

^a, FAO, Food and Agriculture Organization of the United Nations soil classification system

^b, USDA, US Department of Agriculture soil classification system

^c, P₂O₅ used

Table A2. Details of the main findings of the 58 research articles analysed in the present review.

First author	Year	Aim	Main findings
Armstrong, R.D.	2015	P uptake	Addition of P lead to an increment of both wheat shoot growth and P uptake. Addition of P increased N fertilizer recovery, although tillage practices had no effect
Bailey, K.L.	1998	P placement	P fertilizers reduced root disease caused by <i>Gaeumannomyces graminis</i> var. <i>tritici</i> . No effect on other root rot pathogens. Phosphorus fertilizer should be used with zero tillage to reduce wheat losses from diseases. Use of high seeding rates or wide row spacing does not increase risk of damaging root disease development, but might provide higher grain yields
Barbieri, P.A.	2014	P placement; P uptake; Crop yield	Broadcasted P produced lower P accumulation than deep-banded P only at tillering stage. P rate decreased P recovery efficiency, while no effect for placement method. Deep-banded P depressed root arbuscular mycorrhizal colonization compared with broadcast P applications. P placement method did not affect wheat grain yield, plant P uptake, recovery efficiency
Bhaduri, D.	2014	Soil quality indicators	Use of no tillage combined with two irrigations for wheat has potential to improve soil quality. Several indicators analyzed (including available P) might quickly provide warning against soil quality deterioration
Bolland, M.D.A.	2006	Fertilization	Concentrations of P higher when: (i) the element were either deep banded below the seed while sowing, or (ii) the soil was cultivated before drilling the fertilizer with the seed. Deep banding treatment suggests that wheat roots accessed more P from freshly applied fertilizer placed deeper in soil. Mixing fertilizers applied

			using no-till through top 10 cm of soil before drilling fertilizer with the seed in the current year increased plant uptake of soil elements
Brennan, R.F.	2013	Crop yield; soil P	Applying fertilizer when not required had no effect on grain yield, while enhanced P status of soil increased risk of run off, water contamination and subsequent eutrophication
Brye, K.R.	2007	Fertilization; Crop yield	Differing yield responses between locations linked to combination of P and K fertility differences. Detailed information of soil fertility conditions (i.e., extractable P and K) necessary to calibrate correct N doses for wheat crops
Buchanan, M.	1994	Soil P	Significant seasonal P fluctuations detected in all analyzed cropping systems. Fluxes magnitude and quantity of P tended to be higher in reduced-chemical-input systems due to tillage and incorporation of crop, weed, and legume residues. P turnover through microbial biomass with reduced chemical input to soil might be higher if compared to no-till system
Calegari, A.	2013	Tillage; Soil nutrient	Soil P availability more favorable to crop growth up to 10 cm in soil under NT than in CT. P availability higher below 10 cm depth in CT compared to NT. After 19 years of no soil disturbance (NT system) available P content below 10 cm soil layer lower than optimal content of available P recommended. When no cover crops used in winter period, lower biomass resulted in lower P availability in soil
Campbell, C.A.	2011	Tillage; Soil P	Lack of P fertilizer, coupled with export of P in grain and hay, gradually diminished available P and probably suppressed wheat grain yields in later years
Chen, W.	2009	Fertilization	More work might be needed about crop responses to fluid P fertilizer, in particularity on low pH soils. To understand long-term performance of fluid P fertilizer an assessment of its residual value is required, compared to granular P fertilizer
Covacevich, F.	2008	P placement; Arbuscular mycorrhiza colonization	Phosphorus fertilization increased soil P content at 0-10 cm depth as well as plant P content. At two soil depths (0-10;10-20 cm), fertilization with 25 and 50 kg P ha ⁻¹ depressed arbuscular mycorrhiza and arbuscules content if compared to unfertilized treatments, mainly when P was banded. Mycorrhizal colonization negatively associated with soil and plant P contents
Coventry, D.R.	2011	Fertilization; Crop yield	Wheat yield might be limited by soil P availability. Application of farmyard manure in addition to recommended NPK fertilizer rates had effect on yield responses in wheat-rice regions. Grain obtained from NT showed higher protein, grain hardness, and chapatti score compared to other rotations

Cubilla, M.M.	2007	P calibration	Building up (increase) of P in no-till systems more influenced by initial P level than by soil texture. Initial P level affects building up of P in no-till systems, while texture did not show any significance
Daroub, S.H.	2001	Tillage; Soil P	Adoption of no-tillage systems in rotation for 7 years did not increase organic P significantly in any fractions extracted. The use of cover crops did not accumulate organic P
Divito, G.A.	2010	P fertilization; P uptake; Crop yield	No crop yield differences found between annual and rotation fertilization strategies. Control crop (P0) differed from fertilized crops during rotation wheat/soybean. No differences found between crop rotation P use efficiency between nutrient applications. No differences found in applied P recovery efficiency for rotation fertilized treatment than annual application
Essington, M.E.	2000	Tillage; Soil P	No variation of total P observed with depth, but found to be greater in no-till than in disk-tillage system. Organic P found to be higher in no-till system plots. In general, influence of tillage on P distribution limited to soil surface, with few exceptions
Feiza, V.	2010	Soil physical properties; Soil chemical properties; Weed incidence; Tillage	No-till showed higher soil total N, available P ₂ O ₅ and K ₂ O stratification in soil during crop rotation period compared to reduced tillage and conventional tillage. Effectiveness of moderate rate of NPK fertilizers on loam or sandy-loam soils highest in conventional tillage and lowest in no-tillage. Application of high rate of fertilizers on loam not effective in conventional and reduced tillage systems.
Fontoura, S.M.V.	2011	Fertilization; Crop yield	Water soluble fertilizer found to be more efficient compared to phosphate rocks in soils under no-till in both short and long term. Application of phosphates for high yields in soils with high available P contents under no-till unnecessary
Grigoras, M.A.	2013	Crop yield; Tillage, Fertilization	No-tillage system decreased efficiency of fertilization in wheat yield compared to conventional tillage system
Halvorson, A.D.	1992	Crop yield; P fertilization; P placement	Broadcast P fertilization prior to seeding enhanced wheat grain yield
Herrera, W.F.B.	2016	Crop yield	Cumulative grain yield increased proportionally to P dosage. However, higher agronomic efficiency observed when complexed P used
Iqbal, M.	2011	Tillage	Soil P concentration not significantly affected by mulch application, but significant effect of tillage combined with mulching. Soil depth did not have any significant effect on P concentration. Greater P concentration observed in soil upper layers

Jackson, G.D.	1993	Crop yield; P fertilization; P placement	When P soil tests greater than 12 mg kg ⁻¹ , grain yield responded to P fertilization
Jackson, G.D.	1997	Crop yield; soil P	16 mg kg ⁻¹ confirmed as current critical level of soil P for higher grain yield
Karamanos, R.E.	2013	Tillage; Crop yield	Under direct seeding, agronomic efficiency higher when P fertilizer placed in-row rather than mid-row
Kelley, K.W.	2007	Crop yield; P placement	Grain yields influenced by placement of fertilizer. Application of fertilizer in subsurface might be more productive
Kushwa, S.S.	2016	Soil P	Wheat residue on soil surface increased P availability compared to common practice of residue burning. Available P decreased with soil depth, most available P accumulation between 0-5 cm layers
Lafond, G.P.	2001	Fertilization; Crop yield	No grain yield response to P on grain yield when fertilizer N side-banded with P at seeding was detected. Response to P observed when N broadcast in spring. Positive yield responses to P corresponded to soil test (0–15 cm) P levels of 24 kg ha ⁻¹ . No P response observed for soil P levels >34 kg ha ⁻¹ . In general, grain protein concentration not influenced by rate of P fertilizer
Liu, C.A.	2013	Fertilization; Crop yield	Soil available P increased rapidly with time in treatment with manure, N and P and was significantly higher. No significant correlations found between available P and yield or water use efficiency of spring wheat
Loke, PF	2013	Crop yield	No-tillage and stubble mulch increased P compared with ploughing especially in surface layers where crop residues accumulate. Higher P values observed also in no-tillage in comparison with ploughing and stubble mulch.
Lutcher, L.K.	2010	Fertilization; Crop yield	An initial soil test with phosphorus level <12 mg kg ⁻¹ showed consistent P uptake, spikes per unit area and grain yield response. Application of 15 kg ha ⁻¹ P rate can be accepted.
Malhi, S.S.	2008	P placement; Production	Yield differences of flax in rotation with wheat occurred due to P placement. Seed-placed P can reduce seed yield compared with side-banded P
Mooleki, S.P.	2010	Fertilization; Crop yield; P placement	No differences in measured plant variables observed between seed-placed P and side-banded P when averaged over 12 site-years.
Mrabet, R.	2001	Tillage; Fertilization; Soil chemical quality	Soil P content higher in no-till compared with conventional tillage. No-till positively affected extractable P and other soil characteristics (e.g., soil organic matter) in upper root-zone. Wheat in no tillage system might receive more nutrients from decomposition of organic matter and can stimulate soil acidification in seed zone

Noack, S.R.	2014	Soil P; P uptake	Incorporation of crop residues increased rate of release, decomposition and potential for plant roots to access P. Residue P requires more time to break down when retained on soil surface. Analyzed system provided significant amounts of P to subsequent crops.
Parsons, K.J.	2007	P uptake	No significant differences in wheat grain or tissue concentration of P. Inorganic treatment showed higher P removal than all manure treatments. control parcel removing the least
Piegholdt, C.	2013	Tillage	Tillage treatments had little and/or insignificant effects on total soil P content. However, slight increment of soil P content under no-till compared to conventional tillage treatments.
Pradhan, P.R.	2011	Tillage; P uptake; P forms	Organic P content significantly increased in NT system compared to CT irrespective of crop residue application. Reverse trend observed for inorganic P fraction. Crop residue increased Olsen's P content in both CT and NT systems, while tillage systems without crop residue had no effect. Adaptation of zero tillage and crop residue addition for longer duration might require gradual adjustment in recommended doses of P application
Rasmussen, P.E.	1996	Fertilization; Crop yield	P tended to increase grain yield about the same in all analyzed systems. P deficiency did not tend to intensify between late-tillering and harvest
Rasmussen, P.E.	1992	Tillage; Soil P; Crop yield	P deficiency slightly affected by tillage. NT showed lower wheat yields than CT regardless of fertility
Redel, Y.D.	2007	Tillage; Soil P	Tillage and crop rotation exerted same levels of effects on labile+relatively labile P fractions. Labile and relatively labile P fractions both influenced by crop rotation and tillage which in turn mainly affected soil properties. Over fertilization caused both high levels of soluble P and promoted unavailable P accumulation, especially under conventional tillage system
Rehm, G.W.	2003	Tillage; P placement; Crop yield	Impact of P rate on grain yield not affected by tillage system. Rate and placement effects of P fertilizers on P concentration in whole plant tissue did not influence grain yield and dry matter production
Schlindwein, J.A.	2008	P calibration	P concentrations higher in soils under no-tillage in both 0–10 cm and 0–20 cm soil layers
Schwab, G.J.	2006	Tillage; Fertilization; P uptake; Crop yield	No significant differences found between effects of tillage and P management on grain yield of crops grown in soil with available P stratified in 0-15 cm layer. Wheat yields significantly higher in conventional till-system compared to no-till systems

Selles, F.	1999	Tillage; Soil P	Conversion of tilled fallow-wheat system to no tillage continuous wheat showed significant increase in total soil P. This was due to increased accumulation of labile and moderate labile forms of P in surface soil, especially in organic forms
Singer, J.W.	2004	Tillage; Crop yield; Soil P	Soil P concentrations high before establishment of crop rotation in 1998, and unaffected by tillage system
Singh, D.K.	2005	P placement; Production	Rapid drying of soil surface layers reduced availability of soil or fertilizer P influencing response to deep P. Deep fertilizer P remained available during growing season and mitigated P deficiency. Deep placement of fertilizer P significantly affected yield responses
Steiner, F.	2012	Soil P; Fertilization; Crop yield	Application of mineral and organic fertilizers resulted in negative soil P and K balance in the short term. This might jeopardize long-term sustainability of agricultural production system due to depletion of soil nutrient reserves
Thomas, G.A.	2007	Tillage; Fertilization	Soil extractable P (0-10 cm depth) significantly higher in NT than RT or CT. In NT, no significant tillage effects found in extractable P in 10-20 or 20-30 cm soil depths. Higher soil P values found in upper layers of NT probably related to reduced mixing of P fertilizers, increased quantities of organic, and shielding of P adsorption sites
Tiecher, T.	2012	Soil P	Growing crops increases importance of microbial interactions in the P cycle. This effect higher in no-tillage trial, where soil organic P enhanced by amount of crop residues added to soil surface
Tiecher, T.	2012	Tillage, Soil P	Soil inorganic P (labile, moderately labile forms) increased with application of phosphate fertilizer in no-till rows. Soil disturbance in conventional tillage redistributed applied P in deeper layers and increased moderately labile P concentration in subsurface layers.
Tracy, P.W.	1990	Tillage; Minerals properties	Extractable PO ₄ -P concentrations were higher near surface of no-till compared with ploughed soils. Conversely, PO ₄ -P concentrations equivalent below 5-cm depth. No difference in extractable soil PO ₄ -P (0-15 cm) concentrations among tillage treatments
Vieira, R.C.B.	2016	Soil fertility	Considering criteria of fertilized layer (base saturation, available K) and relationship with crop yields (available P), 0.20 cm proved to be most appropriate soil layer for soil fertility evaluation in long-term no-tilled soils
Vieira, R.C.B.	2015	Fertilization; Crop yield	Recommended P fertilization rates for wheat higher than current P fertilization rates normally used. Partially explained by high P adsorption capacity of soils and by high crop yields

Vu, D.T.	2009	Soil P; Tillage	Effects of tillage and crop rotation generally confined to topsoil, with P strongly stratified in topsoil in direct-drill and zero-tillage treatments compared with conventional tillage.
Yuan, Y.	2009	Tillage; Soil properties	Higher soil P levels in watersheds under conservation management attributed to less distribution in soil because of reduced tillage. Spatial relationships of P examined using kriging analysis showed P data spatially dependent and influenced by tillage practices
Zamuner, E.C.	2006	Soil P	To estimate P fertilizer requirements for wheat crops under no-tillage, it is recommended to sample soils before sowing to 20 cm depth. There was strong positive correlation between Bray 1 and Mehlich 3 procedures, thus either could be used to quantify available P in soils with moderate to slightly acidic pH

Chapter II: Phosphorus fertilization, soil phosphorus availability and grain yield of durum wheat (*Triticum durum* Desf.) under no-tillage conditions

1. Introduction

Phosphorus (P) is one of the most important nutrients for growth and development of cereal crops including wheat. It plays an essential role in synthesis of proteins, nucleotides and enzymes, photosynthesis and other physiological and biochemical metabolic activities (Marchner et al., 1993). In recent years, the importance of P to life has focused on attention its efficient use in agriculture for two main reasons: i) the need to maintain or improve P status of agricultural soils, by diminishing run-off and subsequently reduce risk of water bodies eutrophication and ii) the need to prevent depletion of P natural sources (Johnston et al., 2014). Indeed, it is well known that P fertilizers derive from phosphate rock that is a finite, nonrenewable resource. Since P resources are scarce and its extraction is becoming increasingly expensive, it is pivotal to efficiently maximize its use efficiency. In addition, the peak of P use is estimated to occur by 2035, after which the demand would out-strip supply (Cordell et al., 2009).

Nowadays, the efficiency of P fertilization is a challenge facing farmers in both the developed and the developing countries. The essential step is to establish the right rate of P to be applied in field by understanding the crop yield potential and the associated P uptake so to avoid over applications (Roberts, 2005). Some field studies reported that P uptake, in the year of application, rarely exceeds 25% and more often is only 5-10% of the initial amount of P applied (Hinsinger, 2001; Zhu et al., 2012). On the other hand, other studies showed that the residual part of P might become available to future crops beyond the year of application (Roberts 2005; Syers et al., 2008).

The efficiency of P fertilization is even more important in no-tillage conditions where the scarce P mobility negatively affects its homogenous allocation and concentration in depth. According to Calegari et al., (2013), P availability in no-tillage systems is limited to the topsoil with P strongly stratified in the first 0-10 cm layer. In this vision, new P fertilizers based on organo-mineral phosphate complexes were developed with the aim to improving availability and P use efficiency (PUE) to crops (Baigorri et al., 2013; Chen et al., 2004). These new products, namely humic-metal-phosphate are expected to decrease immobilization of P in soil by increasing available amount of soluble phosphate (Urrutia et al., 2014, 2012) so to enhance crop uptake and grain yield (Herrera et al., 2016). These new generation of fertilizers could improve the mobility and reduce the stratification in the first soil layers under no-tillage conditions (Urrutia et al., 2014). Furthermore, some of these organo-P-

complexes are in liquid form being even easier to be up-taken from soil solution compared to the granular ones.

Researches regarding P availability under no-tillage systems and humic-complexed phosphate (HCP) fertilization is still restricted in scientific literature especially concerning wheat cultivated under Mediterranean climate conditions. In this perspective, the aims of this study was to investigate the effect of two different P fertilizers (HCP and triple superphosphate (TSP)) under Mediterranean and no-tillage conditions on i) soil P availability, ii) dry matter synthesis and dry matter translocation iii) P use efficiency and iv) grain yield and grain components of durum wheat (*Triticum durum* Desf.).

2. Materials and methods

2.1. Experimental site

A two-years (2016-2017) field experiment was carried out at the experimental farm “P. Rosati” (43°32'24.5"N, 13°22'30.2"E, Agugliano, AN, Italy) of the Università Politecnica delle Marche. According to Koppen, the climate is classified as Cfa (warm temperate with hot summer) and has an average annual rainfall of 739 mm and a mean temperature of 15°C. The highest mean temperature (25.3 °C) and the lowest mean precipitation (34.2 mm), occurred in July whereas the lowest mean temperature (5.8 °C) and the highest precipitation (94.3 mm), occurred in December (reference period 1981-2010, ASSAM, 2017). Weather conditions of the experimental site, mean temperature and precipitation during the two years of experiment are reported in Figure 1.

The soil is an alkaline-calcareous, silty-clay loam. The soil properties of the experimental plots were analyzed at the beginning of each year of experimentation and reported in Table 1.

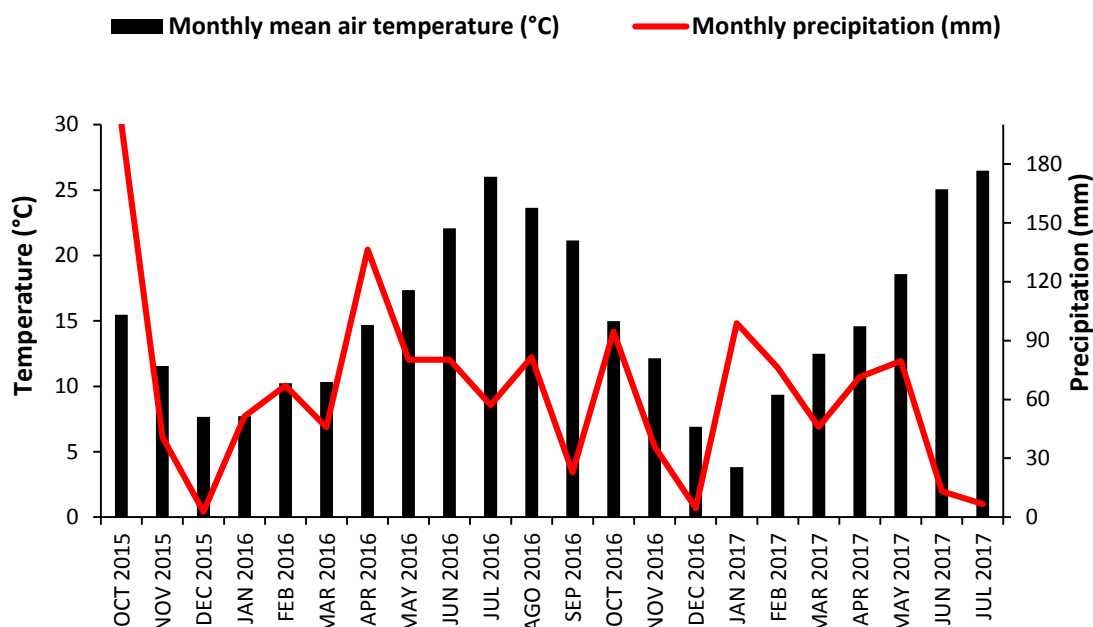


Figure 1. Mean precipitation and air temperature during the experimental period (October 2015 - July 2017).

Table 1: The investigated soil chemical properties (0–20 cm soil layer) of the study sites before fertilizer application in the two years.

Soil chemical properties (0-20 cm)	I year	II year
pH	8.22	8.18
Organic matter (g kg ⁻¹)	16.8	18.8
Available phosphorus (Olsen-P) (mg kg ⁻¹)	9.5	7.7
Available potassium (mg kg ⁻¹)	204	270
Total nitrogen (g kg ⁻¹)	1.1	1.25
Active limestone (g kg ⁻¹)	126	136
Total limestone (g kg ⁻¹)	234	266
Exchangeable sodium (mg kg ⁻¹)	40.0	28
Exchangeable calcium (mg kg ⁻¹)	5467	4751
Cationic exchange capacity (meq 100g ⁻¹)	23.5	15.4
Available Iron (mg kg ⁻¹)	8.6	5.7
Available Manganese (mg kg ⁻¹)	3.3	2.54
Available Zinc (mg kg ⁻¹)	0.43	0.59
Available copper (mg kg ⁻¹)	3.4	1.3
Soluble boron (mg kg ⁻¹)	0.25	0.27
C/N	9.3	8.7
Exchangeable potassium (mg kg ⁻¹)	204	270
Exchangeable magnesium (mg kg ⁻¹)	389	506

2.2. Experimental design

The annual crop rotation was durum wheat (*Triticum durum* Desf.) and maize (*Zea mais* L.). Durum wheat (variety Achille), was sown on November 3rd in 2015 and on November 10th in 2016. The plots were harvested on July 4th in 2015 and July 6th in 2016. The seeding rate was the same in both years 250 kg of seed ha⁻¹. The experiment consisted of five P fertilization treatments with three replicates each, in a randomized complete block design. The experimental plots were 2.5 x 10m.

Fertilization rates were: 1) control (CT), no P fertilization 2) 8.5 kg ha⁻¹ P₂O₅ applied as TSP (PT1) 3) 17 kg ha⁻¹ P₂O₅ applied as TSP (PT2) 4) 8.5 kg ha⁻¹ P₂O₅ applied as HCP, (VP1) and 5) 17 kg ha⁻¹ P₂O₅ applied as HCP (VP2). P fertilizer was applied at seed level during seeding operation by using a precision seed drill.

Nitrogen fertilizer was applied at 160 kg ha⁻¹ (urea), an equivalent dose for each plot, 40% applied at tillering fertilizer and 60% applied at stem elongation. The herbicides application was the same for both years: 5 l ha⁻¹ of glyphosate applied before sowing and 500 g ha⁻¹ of mesosulfuron-metile + iodosulfuron-metil-sodium and 1.5 l ha⁻¹ of florasulame + fluroxipir applied during rising stage. The fungicide and insecticide were applied during flowering stage as prothioconazole + tebuconazole (1 l ha⁻¹) and tau-fluvalinate (1 l ha⁻¹).

The effects of P fertilizers were studied under conditions in which the basic requirements of plants for nitrogen were fully met. Experiments were carried out under rainfed conditions.

2.3. Soil and plant sampling and analysis

A non-systematic (X) pattern (Paetz and Wilke 2005) method was used to collect soil samples from each plot; i.e. a single soil sample, approximately 1 kg, was collected from five sub-samples taken at 0-10 cm and 10-20 cm depth using a manual auger (5 cm diameter). The sampling was performed two months after the seeding. The collected soil samples were gently ground and sieved through a 2 mm sieve. Analyses of soil available P were performed according to Olsen et al., (1954).

At two different growth stages (April 21, May 18 in 2016; April 27, May 18 in 2017), chlorophyll content in the central area of the flag leaf of 30 plants from each plot was determined using a SPAD-502 chlorophyll meter (Minolta, Tokyo, Japan) and expressed as SPAD index (Markwell et al., 1995). The number of plants within an area of 1 m² were cut at ground level and were separated into leaves, culms and ears at wheat anthesis (May 16 in 2015 and May 17 in 2016) to determine dry matter, and into leaves, culms, chaff and grain at wheat maturity stage (June 30 in 2015 and June 28 in 2016), to determine dry matter. An area of 10 m² was harvested to determine grain yield. Yield components such as harvest index, number of ears per unit area, mean caryopses dry weight and number of fertile spikelets and caryopses per ear, were also determined after threshing with a Wintersteiger LD 180 thresher. For dry weight determination, all plant parts were oven dried at 70 °C to constant weight. Protein content was determined through an Infratec 1241 analysis at the Research and Experimental Centre for Crop Improvement (C.E.R.M.I.S), Tolentino (Mc), Italy. Total P content of grain was measured with the ICP-OES method at Agri-Food Service Agency (A.S.S.A.M.) research laboratory, Jesi (An), Italy.

2.4. Dry matter translocation and P use efficiency

The remobilization indices referring to translocation of carbon sources was calculated according to Masoni et al. (2007) and Papakosta (1994):

i) Dry matter translocation ($t\ ha^{-1}$) = dry matter at anthesis – dry matter at maturity [(leaf + culm) + chaff];

ii) Dry matter translocation efficiency (%) = (dry matter translocation / dry matter at anthesis) \times 100;

iii) Contribution of pre-anthesis assimilates to grain (%) = (dry matter translocation / grain yield) \times 100;

iv) Harvest index (HI) = seed yield / total above ground biomass at maturity.

Agronomy efficiency (AE) and PUE indices were calculated according to Xin-kai et al. (2012) and Iqbal (2003): AE (kg grain yield increase per kg P_2O_5 applied) was calculated with the following equation:

$$(AE) = \Delta Y / UP \quad 1$$

Where ΔY is the incremental yield due to P_2O_5 input, and UP is the amount of applied P_2O_5 fertilizer.

PUE (%) was calculated as follows:

$$PUE = \Delta P / UP \quad 2$$

Where ΔP is the incremental P uptake in grain due to P input and UP is the amount of applied P_2O_5 fertilizer. The Y, ΔY , UP are expressed as $kg\ ha^{-1}$.

Statistical analysis

Differences between the treatments were tested using the analysis of variance (one-way ANOVA). The Tukey's test ($P \leq 0.05$) was used to separate the differences among means. Analyses were performed with JMP 11 (SAS) software.

3. Results

3.1. P availability

In general, the available P in the soil was increased with the increasing amount of P_2O_5 applied in the first soil layer (0-10 cm depth) for each year of study (Figure 2 and Figure 3). During the first year of the experiment (205-2016), as compared to the control (CT) available P was increased by 25.5%,

37.9%, 46.4% and 112.4% in PT1, PT2, VP1 and VP2 treatments respectively (Figure 2). Besides, similar trend was partially confirmed during the second year of the experiment (2016-2017). In particular, P availability in PT1, VP1 and VP2 treatments was increased by 5.7, 24.1 and 26.7%, respectively. On the contrary, the soil available P diminished by 18.7% in PT2 and 2.9% in CT. Significant differences were observed for VP2 compared to the all the other treatments with the exception of VP1 (Figure 3).

Lower values were observed at 10-20 cm than those at 0-10 cm depth, nevertheless significant differences emerged between the plots. In the first year of study and in the second soil depth (10-20 cm), the CT and PT2 treatments were increased by 2.3% and 31.1%, while PT1, VP1 and VP2 treatments were decreased by 16.6%, 26.8% and 13,8% respectively compared the same plots in the first soil layer (0-10 cm depth), (Figure 2). In the second experimental season, lower values were observed between the treatments. The plots CT, PT1, PT2, VP1 and VP2 were respectively 83.6%, 96.9, 106.7, 119.2 and 301.5% lower compared the same treatments at 0-10 cm depth and 93.5%, 61.2, 195.6, 39.5 and 178% lower compared the same treatments in the first year of study (Figure 3).

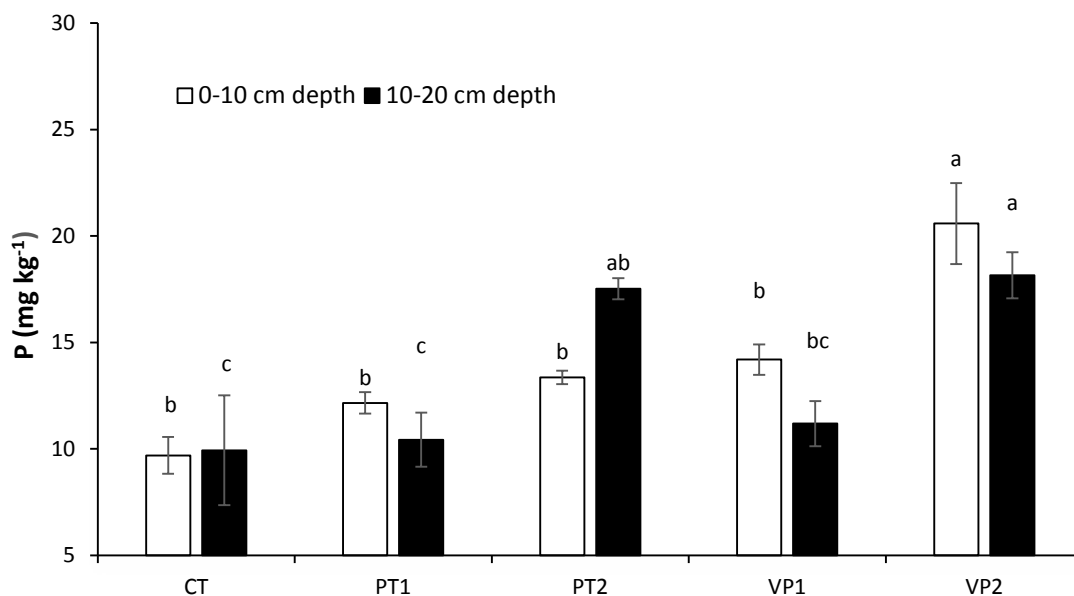


Figure 2. P availability in the first year (2015-2016) of study. Data are mean \pm SE (n=3). Different letters at the same depth stand for statistically significant differences at $P < 0.05$ according to the Tuckey's test.

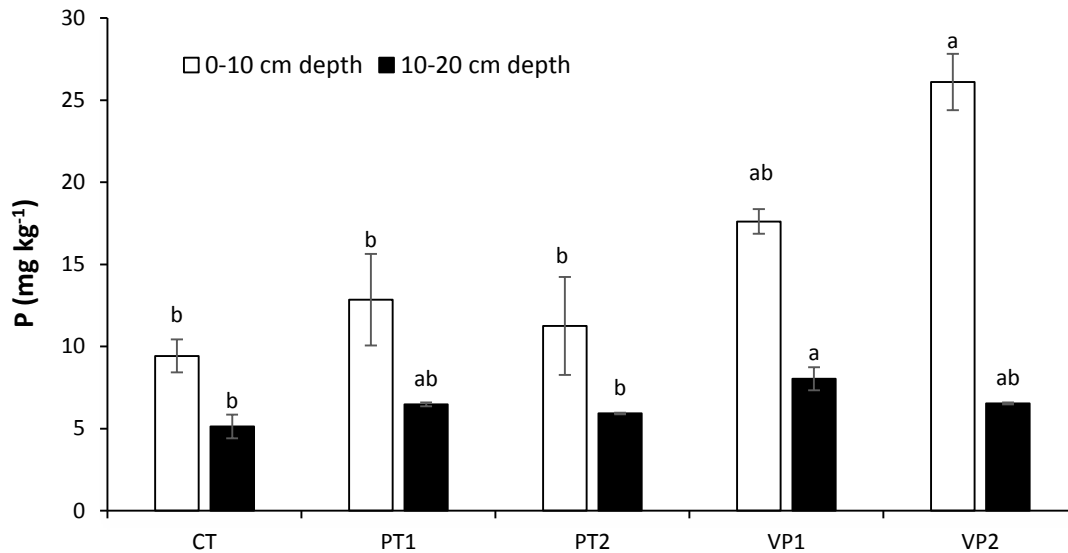


Figure 3. P availability on the second (2016-2017) year of study. Data are mean \pm SE (n=3). Different letters at the same depth stand for statistically significant differences at $P < 0.05$ according to the Tuckey's test.

3.2. Grain yield and yield components

The grain yield and yield components varied considerably between P treatments (Table 2). Marked differences ($p < 0.05$) were reported also between the two years of experimentation especially regarding the grain production. In the first year of experimentation grain yield ranged from 7.9 to 7 t ha⁻¹. The VP2 treatment, showed a higher grain yield (+13.7%) compared with CT. The grain yield of the VP1, PT1, and PT2 treatments were respectively 11.55%, 2.14% and 6.13% higher compared the CT plots even if no significant differences were showed. The grain yield of the second experimental year, ranged from 6.4 to 5.5 t ha⁻¹. The VP1, VP2, PT1 and PT2 treatments were respectively 16.12%, 16.84%, 1.26% and 9.42% higher compared with CT. Despite the discrepancy between the values, the statistical analysis did not highlight any differences ($p = 0.57$).

The number of spikes per unit area ranged from 389.3 to 308 in the first year where VP1, VP2 and PT2 were statistical different form PT1 and CT. The second year showed a higher number of spikes (+25%), the values ranged from 452 (VP1) to 357 (PT1) and there was not significant difference between the treatments. The number of spikelets per spikes was significantly affected by P treatments in each year of study. VP1 and VP2 were respectively 4.02% and 4.97% higher compared PT1 and PT2, and 11.37% and 8.94% compared CT in the first year. The second year experiment highlights a low number of spikelets per spike and higher values were showed for the VP1 and VP2 treatment with 19.7 and 19.6 while the CT was 7.6% lower compared the mean values of the other treatments. No significant differences between P treatments was found for the thousand grain weight (TGW) that ranged from 51.7 (VP2) to 48.8 (CT) in the first year and form 55.6 (VP2) to 50.8 (PT1) the second year.

Table 2. Effects of P treatments on grain yield (12% moisture) and yield components.

Experiment period	Treatment	No. spikes (m ²)	No. spikelets per spike	No. of grains per spike	Grain Yield (t ha ⁻¹)	TGW (g)
2015-2016	CT	322.6 b	20.1 c	56.9 a	7.0 b	48.9 a
	VP1	389.3 a	22.4 ab	64.7 a	7.8 ab	50.4 a
	VP2	368.0 a	21.9 a	61.1 a	7.9 a	51.7 a
	PT1	308.0 b	20.9 bc	56.5 a	7.2 ab	50.9 a
	PT2	381.3 a	20.9 bc	61.4 a	7.4 ab	49.3 a
	<i>F Test</i>	*	*	<i>n.s.</i>	*	<i>n.s.</i>
2016-2017	CT	402.6 a	18.2 b	50.7 b	5.5 a	54.9 a
	VP1	452.0 a	19.7 a	53.1 ab	6.4 a	53.9 a
	VP2	413.3 a	19.6 a	53.3 a	6.4 a	55.6 a
	PT1	357.3 a	19.6 a	51.4 ab	5.9 a	50.8 a
	PT2	396.0 a	19.5 ab	52.4 ab	6.1 a	54.3 a
	<i>F Test</i>	<i>n.s.</i>	*	*	<i>n.s.</i>	<i>n.s.</i>

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test ($p < 0.05$).

3.3. Grain quality

P fertilizer resulted in changes of protein content in grains as shown in Table 3. In 2015-2016, higher fertilizer doses resulted in a higher protein content for VP2 and PT1 compared to CT which reported the lower protein content value (11.7%). A similar trend was observed in the second year of study where only VP2 was significantly different from CT.

Test weight ranged from 85.4 kg hl⁻¹ (PT2) to 86.7 kg hl⁻¹ (VP2) in the first year and from 85.1 kg hl⁻¹ (CT) to 86.2 kg hl⁻¹ (VP1) in the second year. No significant differences were observed between the treatments in both years of experimentation ($p = 0.49$; $p = 0.08$).

Table 3. Effect of P treatments on protein content and test weight.

Experiment period	Treatment	Protein content (%)	Test weight (kg hl ⁻¹)
2015-2016	CT	11.7 b	86.3 a
	VP1	12.5 ab	86.7 a
	VP2	12.9 a	86.0 a
	PT1	12.1 ab	86.3 a
	PT2	12.8 a	85.4 a
	<i>F Test</i>	*	<i>n.s.</i>
2016-2017	CT	12.0 b	85.1 a
	VP1	12.5 ab	86.2 a
	VP2	12.6 a	85.9 a
	PT1	12.1 ab	85.5 a
	PT2	12.2 ab	85.9 a
	<i>F Test</i>	*	<i>n.s.</i>

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test ($p < 0.05$).

3.4. Dry matter accumulation and translocation

Dry matter accumulation was different ($p < 0.05$) between the P fertilization treatments (Table 4). The highest dry matter was found in the P fertilization treatments compared with the control in both years. At anthesis stage, dry matter was higher than the maturity stage (not considering the grains) in all the treatments. However, at harvest, the grain dry matter was similar compared to the vegetative parts (whole plant - grains). After anthesis the total aboveground biomass increased in both years and for all P fertilization treatments. The highest increase in dry matter was found in the VP1 and VP2 treatment in both years of study. Significant dry matter accumulation differences ($p < 0.05$) were found at the first experimental year regarding anthesis and maturity stages. Significant differences at anthesis were found at the second year of study while no significant differences were observed at maturity (vegetative part and whole plant) and in the grains.

Harvest index ranged from 0.46 to 0.48 the first year and from 0.46 to 0.5 the second year of study (Table 5). In the first experimental season, harvest index was not affected by the P fertilization treatments, as the proportion of change in the total biomass and grain yield was similar. Instead, significant difference was found in the second year of study. In particular, the lowest values were registered for CT and PT1 treatments while the highest ones was observed for VP1 treatment.

The dry matter content of vegetative tissues increased between anthesis and maturity in all treatments. In the first year, dry matter translocation of VP1 and VP2 was 26.5% higher in average in the fertilizer treatments compared with CT (Table 5). However, no significant difference was found for the first year ($p = 0.10$). Even in the second year of study, dry matter translocation in PT1 resulted lower compared with CT plots as though the values was not statistically different. The higher value was

observed for VP1 (3.1 t ha⁻¹). VP1, VP2 and PT2 treatments resulted significantly different compared with PT2 and CT.

The dry matter translocation efficiency was highest in VP2 (31.2%) treatment in the first year and in PT2 (33.5%) in the second year. However, this higher dry matter translocation efficiency in VP2 treatment was insignificant (p=0.68; p=0.12). Consistently, the contribution of pre-anthesis assimilates to seed fill was also higher in VP2 the first year and PT2 the second year of study but without significant differences (p=0.64; p=0.27).

Table 4. Dry matter (t ha⁻¹) at anthesis and at maturity (harvest) at the whole plant and grains under different P fertilizer treatments.

Experiment period	Treatment	Anthesis whole plant (t ha-1)	Maturity (vegetative part) (t ha-1)	Grains (t ha-1)	Maturity whole plant (t ha-1)
2015-2016	CT	9.7 b	6.9 b	6.7 b	13.6 b
	VP1	11.6 a	8.2 a	7.5 ab	15.8 a
	VP2	11.4 a	7.8 a	7.6 a	15.5 a
	PT1	9.7 b	7.0 b	7.9 ab	13.9 b
	PT2	10.8 a	7.7 ab	7.1 ab	14.8 ab
	<i>F Test</i>	*	*	*	*
2016-2017	CT	7.5 b	5.7 a	4.9 a	10.7 a
	VP1	9.2 a	6.0 a	6.1 a	12.2 a
	VP2	9.2 a	6.2 a	6.0 a	12.3 a
	PT1	8.1 ab	6.4 a	5.6 a	12.0 a
	PT2	8.6 ab	5.7 a	5.6 a	11.3 a
	<i>F Test</i>	*	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test (p<0.05).

Table 5. Dry matter translocation, dry matter efficiency, contribution of pre-anthesis assimilates to grain and harvest index as affected by the P fertilization treatments.

Experiment period	Treatment	Dry matter Translocation (t ha ⁻¹)	Dry matter efficiency (%)	Contribution of pre-anthesis assimilates to grain (%)	Harvest Index
2015-2016	CT	2.72 a	28.0 a	39.0 a	0.47 a
	VP1	3.32 a	28.7 a	42.6 a	0.46 a
	VP2	3.56 a	31.2 a	44.8 a	0.48 a
	PT1	2.63 a	27 a	36.7 a	0.48 a
	PT2	3.15 a	28.9 a	42.8 a	0.47 a
	<i>F Test</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
2016-2017	CT	1.74 b	23.2 a	32.7 a	0.46 b
	VP1	3.10 a	33.4 a	47.9 a	0.50 a
	VP2	2.96 a	32.4 a	46.8 a	0.49 ab
	PT1	1.68 b	20.7 a	29.6 a	0.46 b
	PT2	2.87 a	33.5 a	48.6 a	0.49 ab
	<i>F Test</i>	*	<i>n.s.</i>	<i>n.s.</i>	*

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test (p<0.05).

3.5. P uptake and P use efficiency

Higher values of P content in grain were observed in VP2 and VP1 for each year of study while CT plots reported the lowest ones (0.36% the first year and 0.39% the second year). Total P uptake (kg ha⁻¹) in grain increased over CT due to P application for each year of experimentation. In the first year, VP1 and VP2 were significantly higher compared with PT1, PT2 and CT treatments. In general, during the second year lower values were observed and no significant difference was recorded between the treatments. However a higher P uptake was found for the VP2 (27,9 kg ha⁻¹) and VP1 (26,3 kg ha⁻¹) treatments (Table 6).

The range of PUE was recorded from 8.6 to 74% in the first year and 20.9-62.1% in the second year. The higher values were found for VP1 treatment in both years of study and the lowest values were observed for PT2 treatment at each year (Table 7).

Agronomic efficiency was higher for VP1 treatment in both years of study. In particular, lower values were found for PT1 treatment in the first year (18.3) and PT2 in the second year (30.1). PT1 was 66% higher compared with PT2 during the second year (Table 6).

Table 6. Effect of P fertilizer on grain yield, phosphorus uptake in grain, P use efficiency (PUE) and agronomic efficiency (AE).

Experiment period	Treatment	Grain yield (t ha ⁻¹)	P in grain (%)	P uptake (kg ha ⁻¹)	PUE (%)	AE (kg ha ⁻¹)
2015-2016	CT	7.01 b	0.36 b	25.5 b	-	-
	VP1	7.82 ab	0.40 a	31.8 a	74.0	95.6
	VP2	7.97 a	0.39 ab	31.6 a	36.0	56.7
	PT1	7.16 ab	0.37 ab	26.7 b	14.6	18.3
	PT2	7.44 ab	0.36 b	26.9 b	8.60	25.4
	<i>F Test</i>		*	*	*	
2016-2017	CT	5.52 a	0.39 b	21.0 a	-	-
	VP1	6.41 a	0.41 ab	26.3 a	62.1	104.1
	VP2	6.45 a	0.43 a	27.9 a	40.7	54.4
	PT1	5.95 a	0.40 ab	23.7 a	31.7	50.0
	PT2	6.04 a	0.40 ab	24.6 a	20.9	30.1
	<i>F Test</i>		<i>n.s.</i>	*	<i>n.s.</i>	

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test (p<0.05).

3.6. Photosynthetic response to P fertilizer (leaf greenness index)

The range of SPAD values determined at the rising and heading (Figure 4 and Figure 5) was 47.3 - 51.1 and 46.9 - 50.8 in the first year and 43.2 - 48.8 and 47.5 - 51.1 in the second year, respectively. During the first year, significant differences were observed between the treatments at rising phase (p<0.05) and heading phase (p<0.05). Statistically different values were also found also at heading stage but not at the rising stage during the second year (p<0.05). In both the years of the study, higher values of SPAD were reported for treatments with HCP fertilizer.

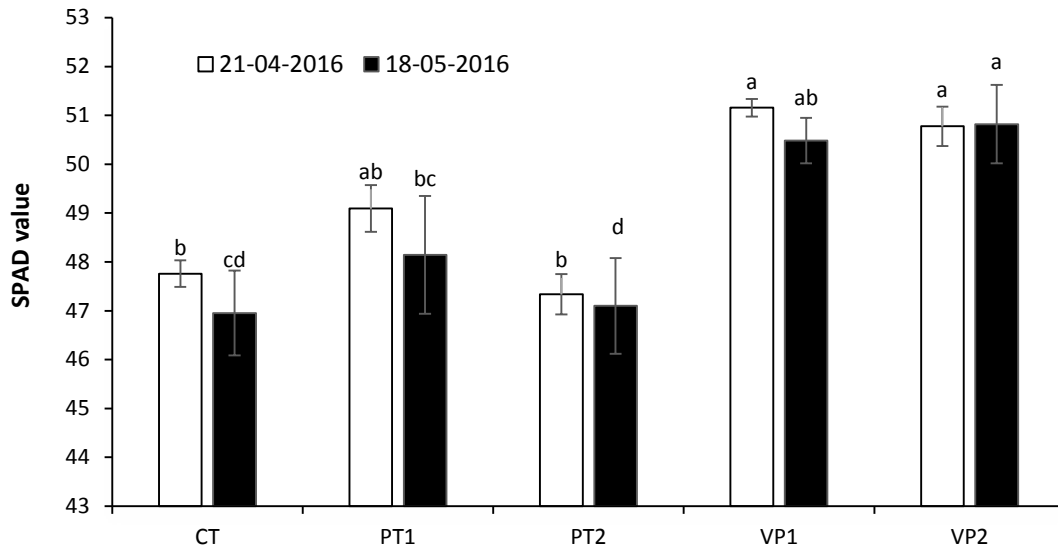


Figure 4. Values of SPAD index related to the first year of study. Data are mean \pm SE (n=3). Different letters at the same sampling day stand for statistically significant differences at $P < 0.05$ according to the Tuckey's test.

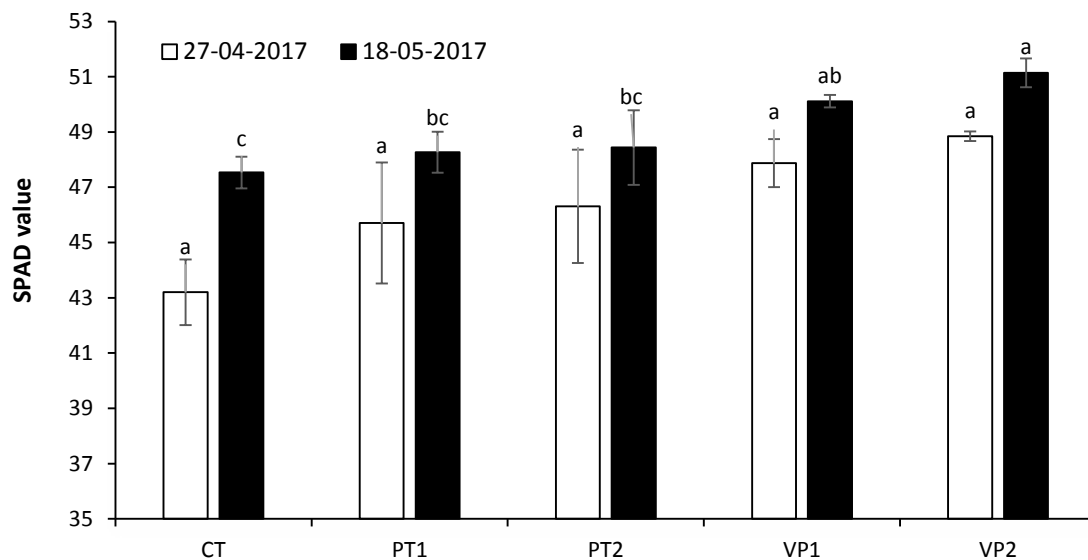


Figure 5. Values of SPAD index related to the second year of study. Data are mean \pm SE (n=3). Different letters at the same sampling day stand for statistically significant differences at $P < 0.05$ according to the Tuckey's test.

4. Discussion

4.1. Soil P availability

HCP fertilizers are relatively new in the market therefore there is a comprehensible lack of studies in the topic. Nevertheless, previous studies investigating the residual cumulative effect of HCP after five consecutive crops (including wheat) and under no-tillage system, reported an higher soil P availability

compared with the standard formulation (single superphosphate) (e.g., Herrera et al., 2016). In this study, the general trend of the soil P analysis suggested that after two months from the seeding, the HCP increased soil P availability more efficiently compared to the TPS. Indeed, as suggested by Urrutia et al., (2012) the theoretical chemical characterization of HCP confirmed the conferred higher P availability and considered this fertilizer as a source that may prevents soil retrogradation through the formation of stable compounds.

The present study highlighted that the soil analysis from 10-20 cm soil layer had a lower P availability (with the exception for the PT2 plots in the first year). This result is consistent with many different studies on P availability (Calegari et al., 2013; Covacevich et al., 2008; Kushwah et al., 2016; Mrabet et al., 2001; Thomas et al., 2007; Vu et al., 2009). The reason of this was attributed to the general poor mobility of P that could be accentuated in no-till soil systems that are characterized by a high soil compaction of the deep layers (Hinsinger, 2001). On the contrary, in the surface layer the higher accumulation of residues and the biological drilling due to roots enhanced the P availability and movement (Herrera et al. 2016; Vanden Nest et al., 2014).

The differences between the two years of study on P availability in the 10-20 cm depth layer (Figure 2 and Figure 3) was linked to the general soil P availability analyzed before sowing. The content of available P showed during the first experimental year was higher compared to the available P founded during the second year of experiment (Table 1). Other reasons may be related to the retention capacity, since its value regulates the amount of water to be delivered into the soil (de Castro et al., 2015). The high precipitations registered in October 2015 (before the seeding) affected the soil capability (through a higher soil moisture) that improved the mobility of P along the second depth layer (Iqbal et al., 2003). However, the liquid formulation of the HCP seems not to had any particular effect on the general P mobility in depth layer.

4.2. Grain yield, yield components and grain quality

In line with what observed by Debiase et al., (2016), crop yield showed remarkable changes due to the variability of climatic factors during the two years. In particular, the lack of precipitations during the second season (2016-2017) especially in the month of June (Figure 1), determined a significant decrease in crop yield. For this reason, the effect of P fertilization between the treatments during the second year was not observed. Despite what observed by Herrera et al., (2016), who concerning grain yield, reported remarkable significances between HCP and the other P treatments, in the present study significant differences emerged only between VP2 and CT during the first year.

The environmental conditions partially influenced the wheat yield components (Ercoli et al., 2017). During the second year, lowest values were observed in terms of the number of spikelets per spike

and number of grains per spike. On the other hand, the climate did not affect the thousand-grain weight and the number of plant per m². Apart from climate, also P treatments affected the number of spikelets per spike, the number of plant per m² the first year and the number of grains per spike the second year and remarkable differences were found mainly between VP2 and CT plots. Similar results were reported by Lutcher et al., (2010), who found significant results in number of plants per m² as result of P fertilization.

It is known that nitrogen taken up before flowering and nitrogen supply during grain filling is fundamental to increase protein content of wheat (Ravier et al., 2017). This study demonstrates the protein content was also slightly affected by the P fertilization since the high amount of P fertilizer enhanced the protein values of the two experimental years. This could be explained by the fact that P may increase nitrogen uptake (Takahashi and Anwar, 2007), since the plants are able to develop an extensive root system enhancing their ability to absorb more nitrogen (Dordas, 2009). The test weight was not affected by the P fertilization and the lower values observed during the second year were attributed to the less favorable climate condition (Ercoli et al., 2017).

4.3. Dry matter accumulation and translocation

Total aboveground biomass increased after anthesis in both years and in all fertilization treatments. A similar effect was found in other plant species such as maize, safflower, winter wheat, and soybean (Dordas and Sioulas, 2008; Koutroubas et al., 1998; Papakosta and Gagianas, 1991). The lower dry matter observed in CT compared to the other treatments was related to the direct link between the lack of P supply that affects the production of photoassimilates and the distribution of assimilates to the reproductive organs (Muchow 1988; Elliot et al., 1997).

In a rainfed environment it is important to maximize the dry matter translocation since it can contribute to higher yield (Cox et al., 1985). Higher values of dry matter accumulation were found for the HCP treatment in both years. In the first year, the grain yield was higher due to the better weather conditions because low rainfall and high temperatures during the grain filling period can have a significant effect on dry matter accumulation (Cartelle et al. 2006; Calderini et al., 2001). In the second year after anthesis no significant differences were observed between the treatments (even in grain yield) because of the lack of precipitation and the high temperature registered after the month of May.

No significant differences were found in the first experimental year concerning dry matter translocation between the treatments. Nevertheless dry matter translocation values were higher in most of the fertilizer treatments compared with the control, suggesting that P fertilization helped plants (mostly during the second season) to translocate higher amount of dry matter (Dordas, 2009).

The contribution of pre-anthesis assimilates to seed can be crucial for maintaining grain yield when adverse climatic conditions reduce photosynthesis, water and mineral uptake (Arduini et al., 2006). Dry matter accumulation during grain filling was lower than dry matter remobilization, so that the contribution of remobilization to grain yield had values higher than 29%. This result is in contrast to what observed by (Masoni et al., 2007) in similar condition who found that most of the dry matter retained in the wheat plant was accumulated before anthesis. The contribution of pre-anthesis storage of assimilates to the seeds (29-48%) are within the range reported by Papakosta and Gagianas, (1991) (6-73%). This can be explained by the central Italy durum wheat flowering time occurring in May when evapo-transpiration reaches high values and a long period of water stress begins. Dry matter partitioning depends on sink number and sink activity and grain number is strongly associated with assimilate availability at flowering (Guitman, Arnozis, and Barneix 1991; Wardlaw, 1990).

The first year of this experiment, the harvest index was not affected by the P fertilization treatments as the proportion of change of the total biomass and grain yield was similar (Dordas et al. 2008; Dordas and Sioulas, in press). Instead, during the second season a decrement in dry matter accumulation was found after anthesis in some plots (VP1, VP2 and PT2 treatments) this permitted to reach a higher harvest index values.

4.4. P uptake and P use efficiency

Significant differences emerged regarding P uptake in grain in the first experimental year. The plots treated with HCP reached high values in each year even if no significance was observed for the second experimental season. This trend confirmed the results of Takahashi and Anwar, (2007) and Iqbal et al., (2003) that found a higher P concentration in the treatments with P application compared the control treatments. The lack of significance in the second year may be related to the climatic condition that affected different parameters as dry matter accumulation (Table 4) and grain yield (Table 2) (Masoni et al., 2007).

It is know that there are different understandings of P use efficiency in wheat in literature, which have resulted in different definitions, such as agronomic efficiency (Alam et al., 2003), first crop recovery efficiency (Cassman et al. 1996; Iqbal et al., 2003; Tang et al., 2008), P fertilizer efficiency (Alam et al., 2003), P use efficiency of shoot or whole plant for dry matter of shoot or whole plant/P uptake amount (Cao et al., 2003), and P physiological efficiency (Dobermann et al. 1998; Tang et al., 2008). In this study, was analyzed the P use efficiency through the values of P uptake in grain and the agronomic efficiency was studied as reported by Iqbal (2003) and Zhu (2012).

In general, highest P use efficiency was observed in the VP1 and VP2 treatments. Between the same type of fertilizer, the higher values were observed for the plots that received less P (VP1 and PT1,

Table 6). This results are in line with Manske et al., (2001) that showed how P uptake efficiency was higher in plots that received less P fertilizer compared the others. A similar trend was observed for the agronomic efficiency where higher rates of fertilizer did not affect the grain yield proportionally. Thus, it was demonstrated that both P use efficiency and agronomic efficiency decrease with the increment of the amount of fertilizer applied. It is therefore important to clarify that regardless the amount of P applied, P use efficiency and agronomic efficiency depend on soil physico-chemical properties, climate and the availability of other major nutrients (Tang et al., 2008).

4.5. SPAD index response to P fertilizer

Phosphorus plays an important role in several physiological processes via photosynthesis, respiration and energy storage (Marchner, 1993; Bakhsh et al., 2008). According to Zhu (2012), P fertilizer may affect the chlorophyll content in the flag leaf especially at the optimal rate. A lack of P results in decrease in chlorophyll content (Jacob and Lawlor, 1991; Machler and Nosberger, 1984) and reduction of photosynthetic capacity of leaf (Lauer et al., 1989). In general, the values of SPAD index obtained in this experiment showed a good plant nutrition similar to what observed by Kulig et al., (2010). During both years of study, significant differences were observed between HCP and the other treatments because P applied as humic-complex affected the chlorophyll content in a better way.

5. Conclusion

The P availability after two months from the seeding increased in treated plots at 0-10cm depth layer and high P availability was observed for HCP treatment at the rate of 17 kg ha⁻¹ P₂O₅ while less availability was reported at 10-20cm depth layer. P fertilization affected grain yield, yield components and grain quality compared the control plots but results were not consistent between the first and the second year of study due to climate conditions. No substantial differences were observed in grain yield between HCP and TSP in both years. HCP affected the dry matter accumulation, dry matter translocation and P uptake more efficiently compared the TSP but no differences in dry matter translocation efficiency were observed. PUE and AE indices demonstrate that the higher rate of P fertilizer had a lower effect on wheat grain yield.

In conclusion, despite HCP conferred a highest soil P availability it affected grain yield as like as TSP in both years of study. This suggest that a high soil P availability is not necessary for an adequate wheat growth and low P fertilizer rate may be sufficient to reach high wheat grain yield in calcareous soil and in Mediterranean conditions.

Nevertheless since some dry matter and yield components parameters resulted positively affected by HCP during the most favorable season (first year) compared with TSP, further investigation are needed to better understand if HCP may result in highest grain yield in better climatic conditions.

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Chapter III: Phosphorus fertilization, soil phosphorus availability and grain yield of maize (*Zea Mays L.*) under reduced tillage conditions in central Italy

1. Introduction

Phosphorus (P) is a primary component of DNA, RNA and ATP and an important carrier of genetic information, which plays a fundamental role to the intracellular energy transfer within living cells. It is also considered as one of the key limiting nutrients for supporting plant photosynthesis and growth (Marschner, 2012). For maize crop, P availability in the soil is an important yield limiting factors in many developing countries, affecting at least eight million hectares of production globally (Lynch, 2011).

The applied P can be easily fixed or adsorbed by soil particles in both acid and calcareous soils. Unfortunately, plants can only absorb a small proportion of applied P (I. C. R. Holford, 1997; Schachtman et al., 1998). In addition, the diffusion rate of P in soils is very low, with the diffusion coefficient of 10^{-12} to $10^{-15}\text{m}^2 \text{s}^{-1}$ (Schachtman et al. 1998). The poor mobility of soil inorganic P is due to different reasons: the first is the conversion to organic compounds by microorganisms; the second is the interactions with cations with the formation of sparingly soluble compounds (Hinsinger, 2001). For these reasons, P fertilization became a fundamental practice to ensure high yield production for human food supply in many countries. However, P is a non-renewable resource and it is important to not exceed the P supply and understand the P uptake requirements of maize crops in order to enhance grain yield without any environmental risks (Xia et al., 2013; Li et al., 2011; Zhang et al., 2012). At the actual rates of extraction, global commercial phosphate reserves will be depleted in 50–100 years and the remaining potential reserves are more costly to extract and may be of lower quality (Cordell, Drangert, and White 2009; Jasinski, 2013). An excessive amount of P fertilizer was often applied to pursue a higher grain yield. Incorrect P fertilizer recommendation, mislead producers into believing that increasing mineral inputs will produce more grain. It is well known that improvements in grain yield do not require proportional increases in the use of chemical P fertilizers (L. Wu et al., 2015). Especially in maize systems, the knowledge in P removal via the grains should improve assessments of global P balance, and optimize P management with correct fertilizer rates.

In the last years, the issues concerning P fertilizer and phosphate reserves have given rise to new approaches to improving P use efficiency (PUE) in agriculture. New P fertilizers based on

organomineral phosphate complexation were developed with the purpose to improve P efficiency, P movement in soil and reduce P fixation with cations (Baigorri et al., 2013). These new products are also expecting to improve the P uptake by plants through lower fertilizing rates decreasing the risks of P waste (Herrera et al., 2016). However, in the Mediterranean area, researches focused on complexed P fertilizers in maize systems are quite restricted in literature.

It is well known that adequate concentration of P is important to maintain high rate of photosynthesis of maize crops and the P deficiency may reduce total leaf area, plant dry matter and grain yield (Rychter and Rao, 2005; Marschner, 2012). Therefore, the Leaf Area Index (LAI), height, and dry matter (DM) variables can be used to describe the architecture of maize plants and predict growth and yield. (Dente et al., 2008). The reliable estimation of these variables during the growing season may improve planning and management of P fertilization treatments (but also any others fertilization treatments) and could supply the necessary tools to avoid the surpluses valorizing the concept of sustainable agriculture.

In view of the above the aims of this experiment was to i) evaluate the soil P availability conferred by different fertilizer rates under two different type of fertilizers, humic-complexed P (HCP) and triple superphosphate (TSP), ii) study the plants architecture (LAI, height and DM) and iii) evaluate the differences in grain yield to identify the best rate and type of P fertilizer, to ensure high production and a better sustainability of maize under Mediterranean and reduced tillage conditions.

2. Materials and methods

2.1. Site description

The experiments were conducted in 2016 and 2017 in two different sites at *Centro di Ricerca e Servizi Azienda Agraria Didattico-Sperimentale "P. Rosati"* (43°32'24.5"N, 13°22'30.2"E) of Università Politecnica delle Marche, Agugliano (AN), Italy. According to Köppen the regional climate is classified as Cfa (warm temperate with hot summer) with average annual precipitation of 739 mm. Climatic conditions during the two years of experiment are reported in Figure 1. The soil type at the study site is a calcareous with a clay texture. The chemical properties of the 0–30 cm soil layer of the study site are showed in Table 1. The soil samples were obtained using the soil auger method and analyzed before fertilization.

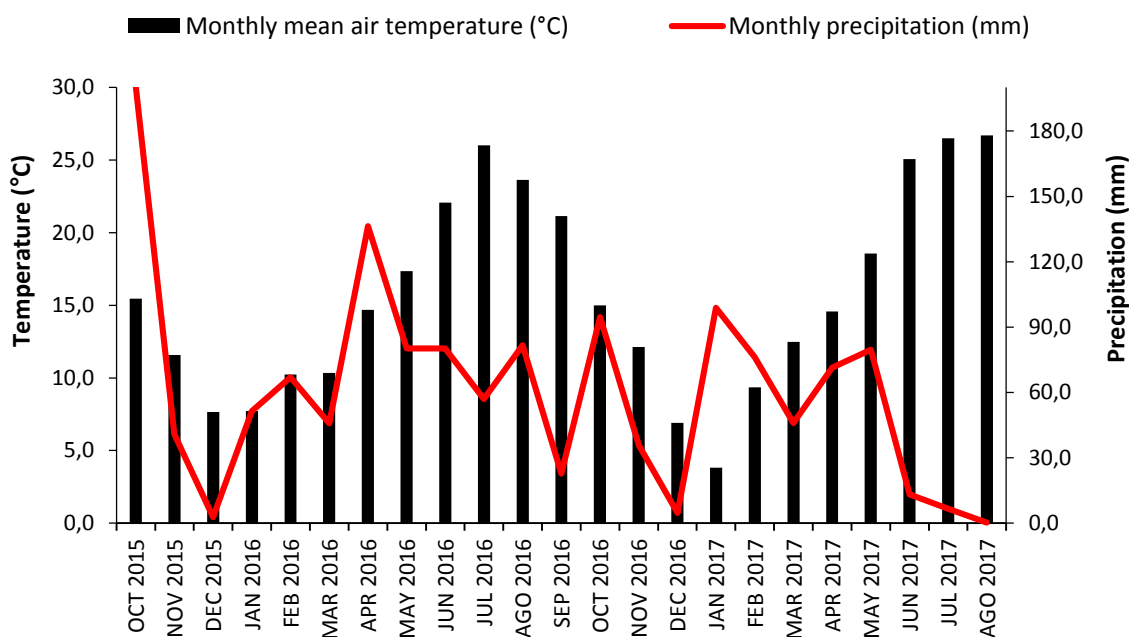


Figure 1. Mean precipitation and air temperature during the experimental period (October 2015 - July 2017).

2.2. Experimental description and design

The annual crop rotation was Maize (*Zea mais* L.) and durum wheat (*Triticum durum* Desf.). Maize seeds were sown on April 20th in 2016 and April 13th in 2017 with the cultivar Monsanto DKC4316. The seeding rate was the same in both years 20.7 kg of seed ha⁻¹. The experiments were carried out under minimum tillage system (chisel plow at 0.25m depth). 92 kg N ha⁻¹ (urea) was applied as pre-sowing base fertilizer. Then 92 kg N ha⁻¹ were banded as top dressing at the V8 stage (the eighth leaf emerged with visible ligule).

The experiments used a randomized block design with three replicates, and each plot was 5 m long and 4 m wide. The distance between rows and plants was 0.5 m and 0.3 cm, respectively. Six fertilization treatments were applied: i) no P fertilization (CT); ii) 11 kg ha⁻¹ P₂O₅ (PT1); iii) 22 kg ha⁻¹ P₂O₅ (PT2); iv) 11 kg ha⁻¹ HCP (VP1); v) 22 kg ha⁻¹ HCP (VP2) and vi) 46 kg ha⁻¹ P₂O₅ (PT3).

Effects of P fertilizer were studied under conditions in which the basic requirements of plants for nitrogen were fully met. Weed growth was controlled by herbicides, the application was the same for both years: 5 l ha⁻¹ of glyphosate applied before seeding and 1.5 l ha⁻¹ of thiencazone-methyl and isoxaflutole applied in May. Experiments were carried out under irrigated conditions with natural precipitation.

2.3. Soil and plant sampling and analysis

One month after the seeding, five random soil cores (0.5 m diameter) from each plot at 0-0.1 m depth and at 0.1-0.2 m depth were collected using 0.05 m diameter metal tubes that were pushed into the soil following a non-systematic (X) pattern (Paetz and Wilke, 2005). Subsamples were air-dried and mixed as one composite sample and then gently ground and sieved through a 2 mm sieve to obtain the air-dried fine fraction. Analysis of soil available P was conducted using Olsen et al. (1954) method.

After 48 days of sowing (DAS) in 2016, 50 DAS in 2017; 64 DAS in 2016, 63 DAS in 2017 and at silking stage (81 DAS in 2016 and 76 DAS in 2017) chlorophyll contents in the youngest but fully developed leaves of 30 plants from each plot was assessed with a chlorophyll meter (SPAD-502, Minolta, Osaka, Japan). The leaf area index (LAI) of each leaf of three plants per plot was measured at silking stage and calculated using a planimeter. For each plot, 20 representative maize plants were also measured using a measuring tape to determine their mean height.

Plants were harvested at silking stage and at physiological maturity (123 DAS in 2016 and 135 DAS in 2017), (when over 50% of the plants showed a visible black layer at the kernel base). At each harvest, six consecutive plants were cut at the stem base. At the second harvest, at maturity, plants were divided into leaves, stems (plus leaf sheath), cobs and kernels.

Plants from an area of 40 m² were harvested to determine kernel yield with moisture adjusted at 15%. 300 kernels from each plot were weighed to determine 100-kernel weight. Harvest index, number of row, length of ear, dry weight and number of kernels per ear were also measured. For dry weight determination, all plant parts were oven dried at 70° C to constant weight. Total P content from grain was measured using ICP-OES method at Agri-Food Service Agency research laboratory (A.S.S.A.M.), Jesi (An), Italy.

2.4. Dry matter increments and P use efficiency

The increments of DM in component organs were analyzed according to Ning et al. (2013). PUE and agronomic efficiency (AE) indices were calculate according to Zhu et al. (2012) and Iqbal et al. (2003) as follow: AE (kg grain yield increase per kg P₂O₅ applied) was calculated using $AE = \Delta Y / UP$, where YP is the grain yield of applied P₂O₅ fertilizer, ΔY is the incremental yield due to P₂O₅ input, and UP is the amount of applied P₂O₅ fertilizer. PUE (%) was calculated using $PUE = \Delta P / UP$, where ΔP is the incremental P uptake in grain due to P input and UP is the amount of applied P₂O₅ fertilizer. The Y, ΔY , UP are expressed as kg ha⁻¹.

2.5. Statistical analysis

The analysis of variance (one-way ANOVA) was performed using the JMP 11 (SAS Inst., 1999) software. The Tukey's test ($P=0.05$) was used to separate the differences among means.

Table 2: The investigated soil chemical properties (0–20 cm soil layer) of the two study sites before fertilizer application in the two years.

Soil chemical properties (0-20 cm)	I year	II year
pH	8.17	8.22
Organic matter (g kg^{-1})	19.1	16.8
Available phosphorus (Olsen-P) (mg kg^{-1})	7.20	9.50
Total nitrogen (g kg^{-1})	1.15	1.05
Active limestone (g kg^{-1})	135	126
Total limestone (g kg^{-1})	253	234
Exchangeable sodium (mg kg^{-1})	19.0	40.0
Exchangeable calcium (mg kg^{-1})	4701	5467
Cationic exchange capacity ($\text{meq } 100\text{g}^{-1}$)	18.1	23.5
Available Iron (mg kg^{-1})	7.70	8.60
Available Manganese (mg kg^{-1})	6.80	3.30
Available Zinc (mg kg^{-1})	0.42	0.43
Available copper (mg kg^{-1})	1.30	3.40
Soluble boron (mg kg^{-1})	0.25	0.25
C/N	9.60	9.30
Exchangeable potassium (mg kg^{-1})	229	204
Exchangeable magnesium (mg kg^{-1})	261	389

3. Results

3.1. Soil P availability

Significant differences in P availability were found between the treatments, in each analyzed layers and in both years of study ($p<0.05$) (Figure 2 and Figure 3). The values of P availability at the 0-0.1 m depth layer ranged from 9.8 to 61.5 mg kg^{-1} in the first year and from 11.4 to 44.3 mg kg^{-1} in the second year. In general, VP1, VP2 and PT3 treatments registered higher P values compared with the rest of plots. Higher values were observed in the 0-0.1 m depth layer in VP2 treatment the first year and in PT3 treatment the second year that were respectively 6.54- and 3.37-fold higher compared to CT. HCP treatments in the 0-0.1 m depth layer were in average 2.02-fold lower in 2017 compared to the values observed in 2016. The treatments PT1 (11 kg ha^{-1} P_2O_5) and PT2 (22 kg ha^{-1} P_2O_5) were not significantly different compared with the control treatments (CT) at each year of study.

The values of P availability in the first year at the 0.1-0.2 m depth layer ranged from 8.62 to 18.08 mg kg^{-1} . The VP2 treatment registered the higher value and was 1.09- and 1.34-fold higher than PT3 and VP1 treatments. PT1, PT2 and VP1 were not significant different compared with CT. In the second year, the values ranged from 8.9 to 17.7 mg kg^{-1} . The higher value was observed in PT3 that was 1.33- and 1.13-fold higher than VP1 and VP2.

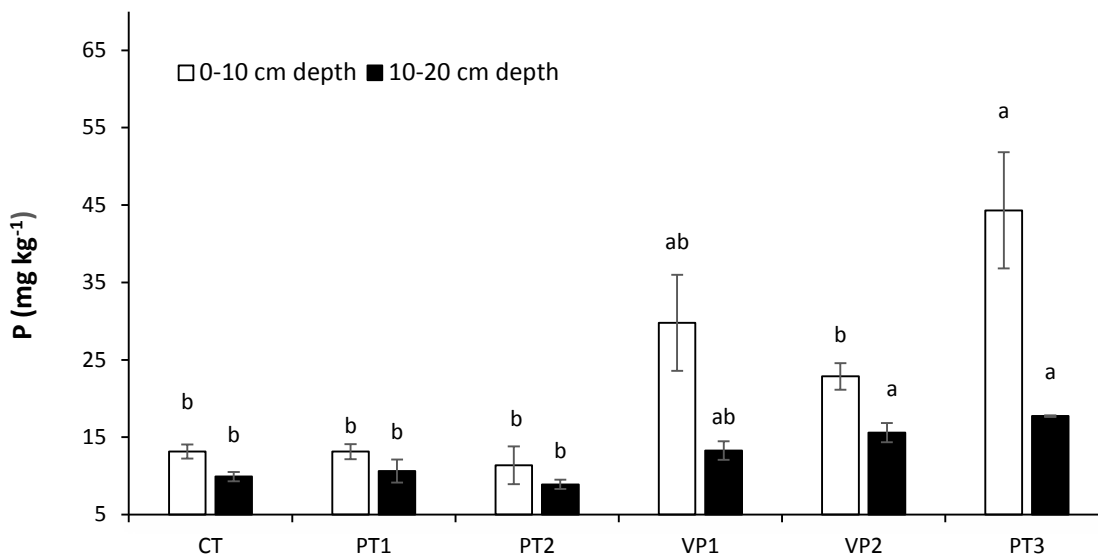


Figure 2. P availability in the first year (2016) of study. Data are mean \pm SE (n=3). Different letters at the same depth stand for statistically significant differences at $P < 0.05$ according to the Tuckey's test.

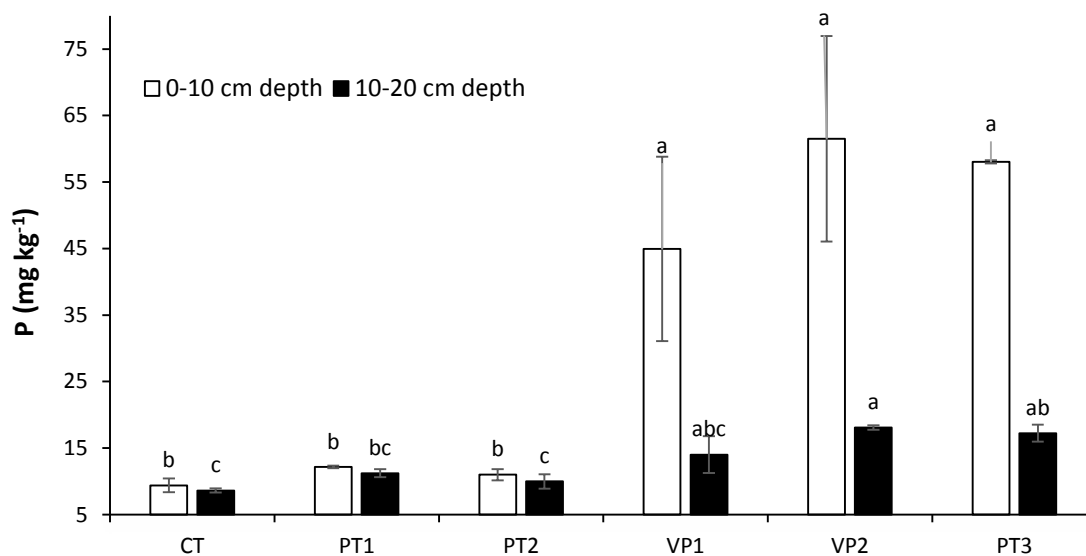


Figure 3. P availability on the second (2017) year of study. Data are mean \pm SE (n=3). Different letters at the same depth stand for statistically significant differences at $P < 0.05$ according to the Tuckey's test.

3.2. Grain yield, yield components and grain quality

Significant differences in grain yields were found among the six treatments (Table 2). During the first experimental year, under the VP2 and PT3 treatments, grain yields were significant different ($p < 0.05$) and increased by 19.3% and 17.2% compared to the CT. Based on the yield components, P fertilization was responsible for an increase in the number of kernel per ear, which, comparing VP2 and PT3 treatments with the CT, were increased by 22% and 28.2%, respectively. The cob length

statistically increased for VP2 treatment that was 8.7% higher compared to the CT ($p < 0.05$). Under the VP1, VP2 and PT3 treatments, 100-kernel weight increased by 4.6, 4.9 and 3.9% compared with CT. There was no significant difference in the number of rows per ear.

During the second year, significant differences ($p < 0.05$) emerged in grain yield that was higher in VP1, VP2 and PT3 treatments (27.5%, 27.5 and 24.1% respectively) compared with CT. Yield components values decreased except for the number of rows per ear (Table 2). The cob length and the number of kernel per ear were higher in the VP1 treatment (17.9% and 16.4%) compared with the CT. There was no significant difference in the number of rows per ear and in the 100-kernel weight.

Grain quality was not affected by P fertilization since no significant differences were found regarding the test weight in both years of study. Nevertheless it was highlighted a general decrease of values (7.6%) during the second year.

Table 2. Grain yield (15% moisture), yield components and test weight under different P fertilizer treatments.

Experimental period	Treatment	Grain Yield (t/ha)	Ear length (cm)	No. Rows per ear	No. Kernel per ear	100-kernel weight (g)	Test weight (kg hl ⁻¹)
2016	CT	9.3 c	19.4 b	15.0 a	450.7 b	28.1 b	80.4 a
	VP1	10.7 ab	20.3 ab	15.7 a	598.1 ab	29.4 a	80.0 a
	VP2	11.1 a	21.1 a	15.5 a	550.3 a	29.5 a	80.6 a
	PT1	9.8 bc	19.5 b	15.6 a	524.2 ab	28.8 ab	80.0 a
	PT2	10.1 bc	19.7 b	15.6 a	510.3 ab	28.5 ab	81.2 a
	PT3	10.9 a	20.5 ab	15.2 a	578.0 a	29.2a	80.5 a
	<i>F Test</i>	*	*	<i>n.s.</i>	*	*	<i>n.s.</i>
2017	CT	2.9 b	16.2 b	15.2 a	356.1 b	21.5 a	75.3 a
	VP1	3.7 a	19.1a	16.6 a	414.7 a	23.1 a	74.0 a
	VP2	3.7 a	17.6 ab	16.4 a	411.1 ab	22.8 a	74.5 a
	PT1	3.3 ab	17.0 ab	16.1 a	386.8 ab	22.3 a	74.8 a
	PT2	3.3 ab	16.9 b	15.8 a	385.0 ab	22.8 a	75.1 a
	PT3	3.6 a	17.9 ab	16.2 a	410.8 ab	22.3 a	75.4 a
	<i>F Test</i>	*	*	<i>n.s.</i>	*	<i>n.s.</i>	<i>n.s.</i>

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test ($p < 0.05$).

3.3. Dry matter accumulation and increment

Post-silking DM accumulation between the treatments in the first year was greater than in the second year (Table 3). The DM gains of the whole plants after silking stage accounted approximately for more than 50% in the first year and less than 50% of the final total DM in the second year. Grains were the largest sink for DM deposition. Significant differences between the treatments were

observed for stems and cob at silking stage and grains at maturity stage, during the first year ($p < 0.05$). VP2 and PT3 treatments registered higher values compared the other plots. No significant differences were observed for the vegetative organs at maturity stage. Compared to the first year, the second year was characterized by similar DM values at silking (for the whole plant on average 115 g/plant the first year and 113 g/plant the second year) and lower DM values at maturity stage (for the whole plant on average 260 g/plant the first year and 147 g/plant the second year). Significant differences were found in the components organs except for leaves ($p < 0.05$). Higher values were observed for the HCP treated plots and PT3 compared PT1, PT2 and CT treatments.

No significant differences emerged regarding the net increment in DM in component organs from silking to maturity stages (Table 4). For each years of study, stems had a negative increment in DM, on average 21.2 the first year and 32.3 the second year. DM increment in whole plant was on average 4.2-fold lower in the second year compared the first year.

Table 3. DM accumulation in component organs of maize at silking (R1) and maturity (R2) stages (g plant⁻¹).

Experimental period	Treatment	Stem R1	Leaves R1	Cob R1	Whole Plant R1	Stem R6	Leaves R6	Cob+Husk R6	Whole Plant R6	Grain
2016	CT	54.4 b	21.5 a	13.4 b	89.3 c	38.7 a	34.1 a	43.9 a	235.3 a	118.5 b
	VP1	71.4 ab	27.0 a	28.2 a	126.5 ab	50.8 a	40.8 a	47.5 a	275.6 a	136.5 a
	VP2	71.3 ab	27.3 a	30.1 a	128.8 a	44.6 a	38.1 a	44.6 a	268.7 a	141.3 a
	PT1	68.7 ab	27.0 a	20.8 ab	116.5 abc	46.9 a	38.7 a	44.4 a	248.7 a	118.7 b
	PT2	58.6 ab	24.6 a	14.6 b	97.8 bc	41.7 a	44.5 a	43.7 a	259.0 a	129.1 ab
	PT3	74.3 a	27.3 a	32.4 a	134.0 a	48.4 a	38.7 a	46.1 a	272.5 a	139.2 a
	<i>F Test</i>	*	<i>n.s.</i>	*	*	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	*
2017	CT	56.4 c	21.7 c	9.8 a	88.1 b	28.9 b	29.5 a	24.5 c	120.3 c	37.3 b
	VP1	73.9 ab	28.4 ab	25.7 a	128.2 a	42.7 a	36.9 a	42.0 a	170.4 a	48.6 a
	VP2	78.3 a	30.1 a	27.6 a	136.2 a	38.5 ab	38.6 a	31.5 bc	157.7 ab	49.0 a
	PT1	66.5 abc	25.6 abc	13.0 a	105.1 ab	32.6 b	31.3 a	31.0 bc	138.8 bc	43.8 ab
	PT2	61.7 c	23.7 bc	15.5 a	101.0 ab	32.5 b	32.8 a	27.7 c	136.5 bc	43.3 ab
	PT3	70.6 ab	27.0 ab	23.9 a	121.6 ab	37.8 ab	36.4 a	36.5 ab	158.2 ab	47.5 ab
	<i>F Test</i>	*	*	<i>n.s.</i>	*	*	<i>n.s.</i>	*	*	*

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test (p<0.05).

Table 4. Net increment in DM (g plant⁻¹) in component organs of maize from silking (R1) to maturity (R2) stages and the percentage of the post-silking DM accumulation to whole plant DM at maturity.

Experimental period	Treatment	Stem	Leaves	Cob+Husk	Whole plant	% of total DM
2016	CT	-15.7 a	12.6 a	30.5 a	145.9 a	61.8 a
	VP1	-20.5 a	13.7 a	19.3 a	149.1 a	54.1 a
	VP2	-26.6 a	10.8 a	14.4 a	139.9 a	51.9 a
	PT1	-21.8 a	11.6 a	23.6 a	132.2 a	53.1 a
	PT2	-16.9 a	19.9 a	29.1 a	161.2 a	62.2 a
	PT3	-25.8 a	11.8 a	13.7 a	138.4 a	50.5 a
	<i>F Test</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
2017	CT	-27.5 a	7.8 a	14.6 a	32.2 a	26.5 a
	VP1	-31.1 a	8.5 a	16.2 a	42.2 a	24.8 a
	VP2	-39.8 a	8.4 a	3.80 a	21.5 a	13.2 a
	PT1	-33.8 a	5.6 a	18.0 a	33.6 a	24.2 a
	PT2	-29.1 a	9.1 a	12.2 a	35.4 a	25.6 a
	PT3	-32.8 a	9.4 a	12.5 a	36.6 a	23.2 a
	<i>F Test</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Negative values denote the net DM export from organs between silking and maturity. Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test ($p < 0.05$).

3.4. P uptake and P use efficiency

No difference was found in the percentage of total P in grains (Table 5), while significant differences emerged between the treatments in P uptake in both years ($p < 0.05$). In general, the values of the second year were 2.6-fold lower than in the first year. VP2 treatment was the highest value in each year of study. Higher values of PUE and AE were found in VP1 in both years of study (Table 5) and regarding TSP treatments, highest values emerged in PT2 the first year and PT2 the second year.

Table 5. Effect of P fertilizer on grain yield, phosphorus uptake in grain, PUE and AE.

Experimental period	Treatment	Grain yield (t ha ⁻¹)	P in grain (%)	P uptake (kg ha ⁻¹)	PUE (%)	AE (Kg ha ⁻¹)
2016	CT	9.3 c	0.20 a	18.6 b	-	-
	VP1	10.7 ab	0.20 a	21.7 ab	37.5	166.6
	VP2	11.1 a	0.20 a	22.2 a	21.1	105.3
	PT1	9.8 cb	0.20 a	18.6 b	0.6	2.4
	PT2	10.1 bc	0.19 a	19.9 ab	7.8	49.0
	PT3	10.9 a	0.21 a	19.5 ab	2.0	35.3
	<i>F Test</i>	*	<i>n.s.</i>	*		
2017	CT	2.9 b	0.22 a	6.5 c	-	-
	VP1	3.7 a	0.24 a	8.9 ab	28.1	98.0
	VP2	3.7 a	0.26 a	9.7 a	18.9	49.0
	PT1	3.3 ab	0.22 a	7.4 bc	10.3	52.2
	PT2	3.3 ab	0.23 a	7.5 bc	5.8	22.8
	PT3	3.6 a	0.24 a	6.9 c	0.8	15.7
	<i>F Test</i>	*	<i>n.s.</i>	*		

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test (p<0.05).

3.5. Plant architecture parameters

Leaf area index (LAI)

At silking stage the P fertilization resulted in significant differences of LAI in both years of study (p<0.05) (Figure 4). In 2016, significant differences were observed for VP1 and VP2 treatments that were respectively 1.29 and 1.27-fold higher than CT. PT treatments were not significantly different from VP1, VP2 and CT. In 2017, was observed a general lowest trend of values. In average LAI decreased of 10.9%. Significant differences emerged for VP1, PT1 and CT (p<0.05).

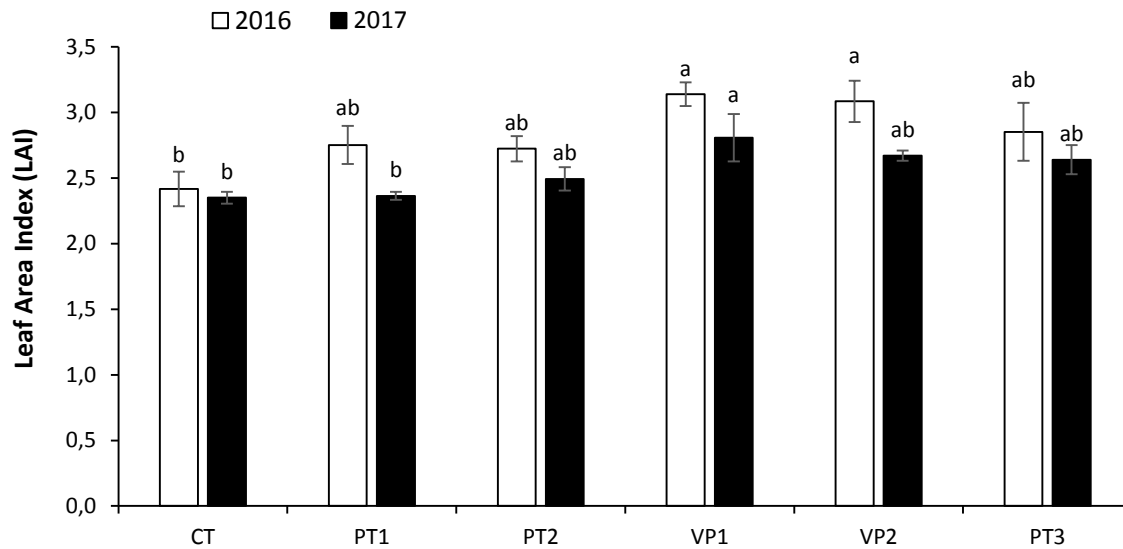


Figura 4. Leaf Area Index (LAI) at silking stage in the two years of study. Data are mean \pm SE (n=3). Different letters in the same year stand for statistically significant differences at $P < 0.05$ according to the Tuckey's test.

Plant heights

In the first year, maize height ranged from 0.18 to 0.23 m at 49 DAS, from 0.60 to 0.94 m at 65 DAS and from 1.92. to 2.07 m at 82 DAS. Higher values were found always for the VP2 treatment that was ever significantly different form PT1, PT2 and CT. 82 DAS correspond to maize silking stage where VP2 treatment was 0.78, 1.56 and 7.91% higher than VP1, PT3 and CT. In general, during the first year, the differences between the plots slightly decreased toward silking stage. In the second year, lowest height values were observed during the three sampling. The range of values start from 0.16 to 0.22 m at 57 DAS, from 0.6.6 to 0.95 m at 76 DAS and from 1.34 to 1.52 m at 88 DAS. At silking stage VP1 was higher compared the other plots (Table 6).

Table 6. Maize heights at three different growth stages in the two years of study.

Treatment	Plants height (cm) - 2016			Plants height (cm) - 2017		
	49 DAS	65 DAS	82 DAS	57 DAS	76 DAS	88 DAS
NT	17.8 d	59.8 c	192.1 c	16.2 c	65.6 c	134 c
VP1	20.2 bc	80.1 b	205.7 ab	18.8 b	80.6 b	151.5 a
VP2	23.0 a	93.6 a	207.3 a	22 a	94.8 a	149.6 a
PT1	17.3 d	61.8 c	193.5 bc	16.8 c	67.7 c	141 ab
PT2	18.1 cd	59.8 c	201.2 abc	16.9 c	67.9 c	137.3 bc
PT3	22.4 ab	87.1 ab	204.1 abc	21.1 ab	93.9 a	148.6 ab
<i>F Test</i>	*	*	*	*	*	*

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test ($p < 0.05$).

SPAD index

During the first year, the values of SPAD index ranged from 53.4 to 58.7 at 58 DAS, from 51.9 to 57.6 at 89 DAS and from 52.1 to 57.9 at 98 DAS. In the second year SPAD index ranged from 49.9 to 54.4 at 67 DAS, from 41.1 to 48.4 at 79 DAS and from 41.3 to 47.9 at 88 DAS (Table 7). Significant differences were observed at each sampling in both year of study. On average, all the values in the first growing season ranged from 54.9 to 56.5. A decrease of values affected maize crop in the second growing season at the 79 and 88 DAS, in fact the range of values was on average 45.6-52.4. Lower SPAD values were found always for CT plots at each year of the experimentation. Higher values were observed for VP1, VP2 and PT3 treatments.

Table 7. Maize SPAD values at three different growth stages in the two years of study.

Treatment	SPAD values - 2016			SPAD values - 2017		
	58 DAS	89 DAS	98 DAS	67 DAS	79 DAS	88 DAS
NT	53.4 c	51.9 c	52.1 c	49.9 b	41.4 b	41.3 b
VP1	57.2 ab	56.4 ab	57.3 ab	54.3 a	48.1 a	47.5 a
VP2	58.7 a	57.2 a	57.9 a	54.4 a	48.4 a	47.9 a
PT1	54.3 c	52.6 c	54.5 c	50.7 b	44.5 ab	45.2 ab
PT2	55.7 bc	54.2 bc	55.5 bc	51.7ab	45.4 ab	46.1 a
PT3	58.1 ab	57.6 ab	57.1 ab	53.8 a	46.6 a	45.8 a
<i>Test F</i>	*	*	*	*	*	*

Asterisk indicates significant differences at 5% level while “n.s.” indicate not significant differences. Different letters in the same column and in the same experimental period stand for statistically significant differences according to the Tuckey’s test ($p < 0.05$).

4. Discussion

The study reported in this paper was affected by unfavorable climate condition since many changes in the climate variables were found during the second year. Seasonal climate changes (high temperature and lack of precipitations) coincided with the most vulnerable stage or flowering period of maize; particularly, a shift in the rainfall pattern generates a water deficit that affected several studied variables (e. g. Mastachi-Loza et al., 2016; Kvaternjak et al., 2015) (Figure 1). It is well known that water stress can affect growth, development and physiological processes of maize, with eventually important reduction in grain yield and biomass (Payero et al., 2008). Several studies have suggested that maize yield is proportional to seasonal evapotranspiration or transpiration (Djaman et al., 2013). This implies that negative transpiration anomalies are a good predictor of lowest productions (Klocke et al., 2004; Stone, 2003; Schneekloth et al., 1991).

In the present paper, higher values of soil P availability after one month from the application were found for the rates 11 and 22 kg P ha⁻¹ for the HCP treated plots and 46 kg P ha⁻¹ for the TSP treated plots (Figure 2 and Figure 3). Furthermore, no statistical differences emerged between the HCP thesis and the highest rate of TSP (46 kg P ha⁻¹). These results showed how the HCP conferred a higher P availability in the soil compared to the TPS treatments. The complexed organic sources are relatively new in the market and there is a lack of scientific literature investigating these fertilizers, however it is possible to find some comparable results. For example Herrera et al. 2016 confirmed the higher soil P availability in the plots treated with HCP fertilizer compared with other fertilizers. Recent studies (e.g. Erro et al. 2012, Herrera et al. 2016) highlighted that complexed P fertilizer were more efficient than the standard P products, in providing available P for plants cultivated in various soils with different physicochemical features. This fact is associated with the ability of complexed P fertilizers to inhibit phosphate fixation and retrogradation in soil, through the formation of stable hetero-ligand compounds (Erro et al., 2012; Urrutia et al., 2012).

In the present field condition, responses regarding grain yield and yield component showed that P fertilization is essential to improve maize grain yield (Arif et al., 2017; Bai et al., 2013; Chen, Liu, and Mi, 2012) in fact the production of the control treatment was lowest in both years of study (Salvagiotti et al., 2017). The lack of difference between VP2 and PT3 (in particular during the first year of study) highlighted that the high rate of P (46 kg P ha⁻¹) was not necessary to reach high yield. The slight differences in grain yield between the treatments (Table 2) was confirmed by Plénet et al., (2000), who studied maize growth under different P application rates. During the two-years experiment, P fertilization affected also the ear length, the number of grain per ear and the 100-kernel weight (Table 2), those results were confirmed by Chen et al., (2012) who studied varietal differences

in P uptake between two Maize inbred lines in alkaline soil. No differences was reported for the number of rows per ear as confirmed by Plénet et al., 2000 who observed that some yield components variables was not affected by P fertilization.

Regarding DM accumulation and partitioning, Jan, Khalil, and Jan, (2010) found that DM partitioning to leaf, ear and stem was higher with the highest P level compared to the lower P levels. The possible reason could be that higher dose of phosphorous enabled the plants to absorb greater amount of the applied P resulting in more assimilate formation and partitioning. Thus, it can be said that P application increased DM accumulation in maize (Arya & Singh, 2001). Regarding the comparison between the two different types of fertilizer showed in the present study, higher values was found for the HCP treated plots and PT3 plot (Table 3). In this regard, Erro et al., (2012) evaluated HCP with TSP formulations in wheat grown in two soils with contrasting pH and different organic matter content and observed the effect of fertilizer source where HCP showed higher DM compared to TSP. Andrade et al., (2014), studied the relations between humic acids and the increase of soil P availability in maize crop and found that the application of humic acid in the soil increase the DM of maize. These results confirmed that HCP fertilizer increase the DM content of maize in a better way compared the TSP as reported in the present study. As showed in Table 4, the DM increments from silking to maturity stage were not affected by P fertilizers in fact were not highlighted any differences with the CT furthermore a small post-silking DM reduction of vegetative organs was showed. This result explain that all of the post-silking DM gains are contributed to grain formation and the most of the grain DM in maize came from the photosynthates produced during grain filling (Ning et al., 2013).

P fertilization also affected the plant architecture parameters (LAI, Height, SPAD index) since significant differences were found between the treated plots and CT (Figure 4, Table 5, Table 6). Some authors (Jan et al., 2010; Plénet et al., 2000) found that LAI showed a positive association with P application. An increment of leaf area per plant with P fertilization was due to the increment of soil P availability that had positive impacts on plant growth. In the present study, a highest LAI was reported for HCP treated plots (Figure 4), this could be explained also in this case by the highest soil P availability conferred by HCP fertilizer. Jan et al., (2010) found also a positive association between P fertilization and plant height. In this experiment, P application affected the plant heights and significant differences were mainly observed between PT3, HCP treated plot and CT (Table 5). Regarding SPAD index, the visible significant differences between the treated plots and CT explained that P application can affect the chlorophyll content in the leaves. Jing et al., 2010 who studied localized application of P on maize crop, confirmed this result.

Significant differences emerged regarding P uptake (kg ha^{-1}) in grain in the first experimental year (Table 5). The VP2 plot reached high values in each year. No differences were found between TSP treated plots and CT. It can be said that a highest soil P availability (conferred by HCP treatments) increase the P uptake in grain. This trend confirmed the results of Kihara and Njoroge (2013), Messiga et al., (2012) and Iqbal et al., (2003) who found a higher P concentration in the treatments with P application compared the control treatments. In the present paper, P use efficiency through the values of P uptake in grain and the agronomic efficiency was studied as reported by Iqbal et al., (2003) and Zhu et al., (2012). The highest P use efficiency was observed in the VP1 and VP2 treatments. Regarding HCP treatments, the plot with lowest applied P reached highest PUE values, similar trend was observed for TSP treated plots. The result confirmed what had found Kihara et al., (2013) that considered low amount of P fertilizer applications as more efficient and more economical. This results are also in line with what found Manske et al., (2001) in wheat, the crop registered highest level of PUE with low rates of P fertilizer. Soil P availability affected also the agronomic efficiency of P fertilizers. In the present study, higher values were found for HCP treated plots compared the rates of P fertilizer applied as TSP that did not affect the grain yield proportionally. It was also showed that lowest rates of P fertilizer conferred highest levels of AE (Kihara et al., 2013; Messiga et al., 2012; Iqbal et al., 2003).

5. Conclusions

Highest doses of P applied as TSP (46 kg ha^{-1}) had the similar effect of HCP treatments (11 and 22 kg P ha^{-1}) in dry matter accumulation, LAI and height of plants. HCP fertilizer resulted more efficient compared to TSP and at one month from the seeding affected the soil P availability in a better way. It is important highlight that P fertilization was crucial to improve maize production but high rates of P fertilizer are not required to reach high grain yield in Mediterranean condition and in alkaline soils. The present paper shows that 22 kg ha^{-1} applied as HCP is the best rate of P fertilizer able to reach, high grain yield, PUE and AE and ensure highest environmental sustainability.

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General conclusions

The increasing trend of publications regarding P fertilization on wheat in no-tillage systems over the last years shows that these topics are currently under investigation. However, it is also important to highlight that the limited number of studies in Mediterranean countries, where wheat-based systems are dominant represent gap of knowledge of paramount importance and thus research need to urgently address this issue. From the scientific articles analyzed in the systematic review clearly emerged the need to expand the study areas in which P dynamics are investigated as well as the need to carry out long-term studies to fully characterize P dynamics. Moreover, a site-specific calibration of P fertilization must be implemented in order to avoid over or under-fertilization.

The experimental trials carried out during 2015-2016 and 2016-2017 seasons confirmed the importance of a correct P application rate and how the use of new products like humic-complexed P fertilizers can reduce the P dosage improving the soil P availability, P use efficiency by crops and plant growth. The experimental trials on durum wheat showed that the humic-complexed P fertilizer affected the dry matter accumulation, the dry matter translocation and some yield components parameters more efficiently compared the triple superphosphate. The experimental trials on maize crop in reduced tillage system, showed the importance of P fertilization to improve grain yield and demonstrated that with low rate of P applied as humic-complexed P fertilizer, it can be possible replace high rates of P fertilizer applied as triple superphosphate ensuring a correct plant growth, high grain yield and a better environmental sustainability.

In conclusion, reducing the surplus of P per unit area, improving the efficiency of P fertilizers and discouraging the excessive use of P through the use of new P based fertilizers, can be an important step to postpone the depletion of phosphate rock and improve the sustainability of the fertilization practices. Further researches are needed to better understand if this new generation of P fertilizers can be competitive even in other crops and can completely replace the standard P formulations largely used in agriculture till now.