

**ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES**

MSc THESIS

Alexandra BYKOVA

**EFFECTS OF LONG-TERM PHOSPHORUS FERTILIZER
APPLICATIONS ON PLANT GROWTH, YIELD, SOIL
CARBON BUDGET AND CARBON FLUX**

SOIL SCIENCE AND PLANT NUTRITION DEPARTMENT

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ABSTRACT

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EFFECTS OF LONG-TERM PHOSPHORUS FERTILIZER APPLICATIONS ON PLANT GROWTH, YIELD, SOIL CARBON BUDGET AND CARBON FLUX

Alexandra BYKOVA

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Long-term agricultural researches with annual and perennial cropping systems provide significant insight into the carbon sequestration potential of agriculture in the South-East Anatolia region. This study was exploring an effect of different phosphorous (P) fertilizer application rates on the storage and quality of soil organic carbon (SOC), permanganate oxidizable C (PoxC), plant biomass and carbon uptake, plant CO₂ assimilation rate to assess the relationship between Carbon Sequestration and P fertilizers application. Different cropping sequences of corn (*Zea mays L.*) - wheat (*Triticum aestivum L.*) (CW system) and application of 4 fertilizer doses (0, 50, 100, 200 kg ha⁻¹) were repeated annually during last 20 years. The study was based on the hypothesis that long-term P-fertilization can affect Carbon Sequestration in soil and plant biomass and impacts on soil CO₂ flux. The objectives of the present study were to: (i) analyze the effect of long-term inorganic chemical P fertilization on the plant biomass carbon uptake and SOC amount for wider environmental protection, (ii) estimate effect of long-term P fertilization on CO₂ flux related with SOC accumulation. The results demonstrated that P fertilization has positive impact on plant biomass, higher carbon fixation and more carbon residue left in soil, that in a long perspective has an positive impact on soil carbon sequestration. The P fertilizer did not have direct impact on SOC of summer-maize growing season, and had positive impact on SOC while winter-wheat growing season. The fertilization also did not demonstrated direct impact on soil CO₂ flux.

Key words: Soil organic carbon, Climate change, Carbon dioxide

ÖZ

YÜKSEK LİSANS TEZİ

UZUN SÜRELİ FOSFOR UYGULAMASININ TOPRAK KARBON
SEKESTRASYONU VE ATMOSFERE KARBON DİOKSİT SALINIMI
ÜZERİNDEKİ ETKİLERİ

Alexandra BYKOVA

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Akdeniz bölgesi ekosisteminde tek ve çok yıllık ürün deseninde olmak üzere gerçekleştirilen uzun süreli tarımsal çalışmalar karbon birikimi hakkında önemli bilgiler sağlamıştır. Yürütülen bu yüksek lisans tez çalışmasında karbon bağlanması (Karbon Sequestration) ve fosforlu (P) gübre uygulaması arasındaki ilişkinin belirlenebilmesi amacıyla farklı fosfor gübresi dozlarının, toprak organik karbonu, bitki biyomasına, bitki karbon alımına, bitki CO₂ asimilasyon oranına etkileri araştırmaktadır. 1998 yılında çakılı deneme olarak kurulan ve son 20 yılda mısır (*Zea mays L*) ve buğday (*Triticum aestivum L*) (sürekli buğday) ekim nöbeti içinde 4 farklı fosforlu gübre doz (0, 50, 100, 200 kg ha⁻¹) uygulaması yapılarak araştırma sürdürülmektedir. Mevcut tez çalışması 2016-2017 dönemlerinde yürütülmüştür. Çalışmanın hipotezi, uzun süreli fosfor gübrelemesi toprak ve bitki biyoması olan karbon birikimini etkileyebilir ve topraktan salına CO₂ akışını etkileyebilir üzerine kurulmuştur..Elde edilen sonuçlar fosfor gübrelemesinin kök biyomasında artış, karbon fiksasyonu ve toprakta daha çok karbon artığının kalması ile kapsamlı incelendiğinde toprak karbon birikimi üzerinde pozitif etkisinin olduğunu belirlenmiştir. İkinci ürün mısır bitkisinin ekili olduğu yaz sıcaklarının olduğu dönemde, fosfor doz gübrelemesi ile toprak organik karbon tutulması arasında mineralizasyonun yüksek olması nedeniyle bir ilişki bulunamamıştır. Kış mevsimi-buğday bitkisi yetiştirildiği döneminde ise fosfor gübresi ve toprak organik karbonu arasında istatistiksel bir ilişki bulunmuştur. Aynı zamanda fosfor gübrelemesi toprak CO₂ akışı üzerine her iki bitki içinde doğrudan bir etkinin bulunmamıştır.

Anahtar Kelimeler: Organik karbon, Toprak CO₂ akışı, İklim değişikliği

GENİŞLETİLMİŞ ÖZET

Toprakta organik madde, hem bitki besleme hem de toprak özelliklerinin korunumu ve sürdürülebilirliği açısından önemli bir faktördür. Toprakta organik maddenin bozunumu toprakta gerçekleşen gaz değişimlerinin de bir parçasını oluşturmaktadır ve karbondioksit, metan, azot oksit gibi sera gazlarının salınımına neden olmaktadır. Tarım topraklarında gerçekleşen CO₂ akışı, kök solunumu, bitki artıklarının ayrışması, toprağa ilave edilen bitki artıkları ile ya da köklerde yaşarmaya (eksüdasyon) bağlı ayrışma durumu ve toprak organik maddesinin mikrobiyal ayrışması sırasındaki bazal solunum ile ilgilidir (Kuzyakov, 2006). Fisk ve ark. (2015) tarım topraklarında mineral fosforlu gübreleme uygulamasının sadece fosfor açısından yetersiz topraklarda değil herhangi bir ekosistemde de toprak organik maddesini arttırabildiğini belirtmiştir.

Bu çalışmanın amaçları: (i) uzun süreli inorganik P gübrelemesinin, bitki biyokütlesi ile kaldırılan karbon miktarı ve toprak organik karbon deposunun daha geniş çevresel anlamda koruma için etkilerini analiz edilmesi, (ii) Uzun süreli P gübrelemesinin, toprak organik karbon birikimi ile ilgili olan CO₂ akışları üzerine etkilerinin tahmin edilmesi. Bu çalışmanın hipotezi uzun süreli P gübrelemesinin karbon sekestrasyonu olan toprak ve bitki biyokütlesi ve toprak CO₂ akışlarına etki etmesi üzerine kurulmuştur. Yürütülen bu yüksek lisans tez çalışmasında farklı fosfor gübresi dozlarının, toprak organik karbonu miktarına ve kalitesine, permanganate ile okside olabilir karbon (PoxC) içeriğine, fulvik asit (FA) ve hümik asit (HA) içeriğine, bitki biyokütlesine, bitki karbon alımına, bitki CO₂ asimilasyon oranına etkileri araştırılmıştır. Geleneksel olarak işlenen topraklarda iki farklı bitki olarak mısır (*Zea mays L*) ve buğday (*Triticum aestivum L*) (sürekli buğday) ekim nöbetinde ve 4 farklı fosforlu gübre dozları (0, 50, 100, 200 kg ha⁻¹) yıllık olarak tekrarlanarak 20 yıl boyunca (1997-2017) sürdürülmüştür.

Mısır bitkisi büyüme dönemindeki sonuçlar, fosfor gübrelemesinin mısır bitkisinde gövde, kök ve toplam biyokütle üretimini önemli derecede arttırdığını

göstermiştir. Fosfor gübrelemesi bitki yapraklarındaki karbon içeriği üzerine etkisi olmamıştır, ayrıca bitki kök üstü aksamında azot ve fosfor içeriğini önemli derecede arttırmıştır. Sonuç olarak fosfor gübrelemesi, mısır bitkisi biyokütlesi ile kaldırılan azot miktarını önemli derecede arttırmıştır. Mısır bitkisi biyokütlesinde karbon alınımları ve CO₂ fiksasyonu, fosforlu gübre dozları ile kademeli olarak artış göstermiştir, ancak aradaki ilişki istatistiksel olarak zayıf bulunmuştur. Mısır bitkisi biyokütlesinde en yüksek düzeyde toplam karbon alınımları (10.9 t h⁻¹) P-100 uygulamasında ile en düşük düzeyde toplam karbon alınımları (8.9 t h⁻¹) ise kontrol uygulamasında bulunmuştur. Fosfor gübrelemesinin mısır bitkisinde Net fotosentez asimilasyon oranı üzerinde bir etkisi olmamıştır. Toprak organik karbonu değerleri fosfor gübrelemesinin, mısır yetiştirme periyodunun ardından toprak karbon deposu üzerine olumlu veya olumsuz etkiye sahip olmadığını göstermektedir.

Karşılaştırmalı analizlere göre tüm uygulamalarda her bir yetiştirme sezonu için toplam biyokütle ile CO₂ alınımları, toprakta toplam CO₂ kayıplarından daha fazla gerçekleşmiştir buna bağlı olarak çalışma alanında her bir üretim periyodu için pozitif CO₂ değişim dengesine sahip olduğunu da göstermektedir. En yüksek fosforlu gübre dozları (100, 200), topraktan daha az CO₂ kaybına bağlı olarak mısır bitkisi biyokütlesinde daha yüksek CO₂ tutunumu gerçekleşmesinden dolayı toprak akışlarında daha az CO₂ kayıpları (24.3 ve 22.5 t ha⁻¹) gerçekleştiğini göstermiştir. Kontrol parsellerinde, tavsiye edilen gübre dozundan daha düşük sonuçlar elde edilmiştir. Biyokütle ile CO₂ tutunumu 29.9 t ha⁻¹ ve topraktan akışları ile CO₂ kaybı ise 24.3 t ha⁻¹ ölçülmüştür, böylelikle CO₂ değişim dengesi pozitif bulunmuştur.

Buğday bitkisi büyüme periyodunda fosfor gübrelemesi, buğday toprak üstü aksamı, kökü, dane verimini ve toplam biyokütle üretimini önemli derecede arttırmıştır. Fosfor gübrelemesi buğday bitkisinde yaprak karbon içeriği üzerine etkisi olmamıştır, ancak kök dokularının azot ve toprak üstü aksamın fosfor içeriğini önemli derecede arttırmıştır. Sonuç olarak buğday biyokütlesinin azot alınımları ile fosfor gübrelemesi arasında istatistiksel anlamda olumlu bir ilişki

bulunmuştur. Buğday biyokütlesi ile karbon alınımlı ve toplam CO₂ fiksasyonu olumlu olarak önemli ölçüde etkilenmiş ve fosfor gübresi dozu artışı ile sürekli olarak artış göstermiştir. Buğday biyokütlesinin toplam karbon alınımlında en yüksek değer (11.1 t h⁻¹) P-200 uygulamasında, en düşük değer (6.61 t h⁻¹) ise kontrol alanında bulunmuştur. Fosfor gübrelemesinin buğday bitkisinde Net fotosentez asimilasyon oranı üzerinde bir etkisi olmamıştır. Toprak sonuçları fosforlu gübrelemenin, buğday rotasyonundan sonra toprak organik karbon içeriği üzerine etkisi olduğunu göstermiştir.

Karşılaştırmalı analizlere göre çalışmada uygulanan bütün gübre dozları topraktaki CO₂ dengesini olumlu etkilemiş olup, düşük derecede toprakta akışların olması ve daha fazla biyokütle ile karbon alınımlı nedeniyle mısır bitkisi döneminde önemli derecede yüksek sonuçların olduğunu göstermiştir. Kontrol parsellerinde biyokütle ile CO₂ tutum miktarı 24,2 t ha⁻¹ ve topraktan akış olarak kaybolan CO₂ miktarı da 15.3 t ha⁻¹ olarak ölçülmüştür. En yüksek fosforlu gübrelemenin (200 kg P₂O₅ ha⁻¹) yapıldığı çalışma parsellerinde ise en yüksek biyokütle ile CO₂ tutunumu (40.6 t ha⁻¹) ve en düşük topraktan akışla CO₂ kaybı (15.4 t ha⁻¹) ölçülmüştür; bu sonuçlara dayanarak fosfor gübrelemesi ile biyokütle ile karbon alınımlı (sekestrasyon) üzerine etkisi vardır.

Ekim yapılmamış boş toprak parseli ile ekili toprak parseli arasında CO₂ akışları açısından karşılaştırma yapıldığında, ekili olmayan toprakta önemli derecede daha düşük akışlar gerçekleşmiştir bununla birlikte önemli derecede CO₂ asimile edebilen örtü bitkileri, toprakta CO₂ akışlarını dengelemiştir.

Sonuç olarak: (i) Fosfor gübrelemesi mısır ve buğday bitkisinin bitki biyokütlesi üzerinde olumlu bir etkiye sahiptir ve bu nedenle atmosferdeki CO₂ nin tutunumunu arttırmaktadır; (ii) Fosfor gübrelemesi kış mevsiminde buğday bitkisi yetiştiriciliğinde SOC içeriğini olumlu etkilemiştir, ancak mısır bitkisi yetiştiriciliğinde yaz mevsiminde hiçbir etki göstermemiştir ; (iii) Tüm fosfor gübrelemesi dozları, toprak CO₂ akışı üzerinde ne olumlu ne de negatif etki göstermemiştir. Ancak, ekili arazilerde çıplak toprağa kıyasla daha yüksek CO₂

akışı deęerleri bulunmuştur; (iv) Çalışmada fosfor gübrelemesi, hem mısır hem de buğday bitkisi için fotosentezde CO₂ asimilasyonu üzerine etkili olmamıştır.



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ABBREVIATION

AC	: Active Carbon
CO ₂	: Carbon Dioxide
CWS	: Corn-Wheat System
FA	: Fulvic Acid
FAO	: Food And Agriculture Organization Of The United Nations
Gc M ⁻²	: Grams Of Carbon Per Square Meter
GCC	: Global Carbon Cycle
GHG	: Greenhouse Gases
HA	: Humic Acid
HS	: Humic Substances
IPCC	: Intergovernmental Panel On Climate Change
LOC	: Labile Organic Organic
LOI	: Loss Of Ignition
MBC	: Microbial Biomass Carbon
Mg	: Mega Gram
MRT	: Mean Residence Time
NAR	: Net Assimilation Rate
NOAA	: National Oceanic And Atmospheric Administration Earth System
NPP	: Net Primary Productivity
Pg	: Petagram
Pi	: Phosphorus Inorganic
Po	: Phosphorus Organic
POC	: Particular Organic Carbon
POM	: Particular Organic Matter
Poxc	: Permanganate Oxidizable Carbon
Pt	: Phosphorus Total

Rothc : Rothamsted Carbon Model
SI : Sustainable Intensification
SIC : Soil Inorganic C
SOC : Soil Organic C
SOM : Soil Organic Matter
TOC : Total Organic Carbon
USDA : U.S. Department Of Agriculture



1. INTRODUCTION

In December 2015, UNFCCC-COP21 in Paris, Minister of Agriculture of France, Stephane Le Foll, proposes to increase organic matter contents and encourage carbon sequestration in soils, through the application of appropriate farming and forestry practices and challenged the world to see that agriculture is part of the solution to climate change (UNFCCC, 2015). SOC sequestration is a part to achieving this at the rate of '4 per 1000', particularly means annual increase of "4 per thousand" (0.4%) each year of organic matter in soil would be enough to compensate for the global emissions of greenhouse gases. More than 100 countries pledged to reduce agricultural greenhouse gas (GHG) emissions in the 2015 Paris Agreement of the United Nations Framework Convention on Climate Change (UNFCCC, 2015).

Turkey submitted its Intended National Determined Contribution (INDC) on 30 September 2015, with a greenhouse gas reduction target (including land use, land use change and forestry (LULUCF) of up to 21% below business as usual (BAU) by 2030 (The Republic of Turkey, 2015). Turkey government's plans and policies to be implemented for this INDC completion for agriculture sector are: (I) Fuel savings by land consolidation in agricultural areas; (II) Rehabilitation of grazing lands; (III) controlling the use of fertilizers and implementing modern agricultural practices; (IV) supporting the minimum tillage methods.

Aspects of the possible negative impact of soil and crop management such as fertilizers use on soil carbon, nowadays it is became a critical topic in agricultural research area. With environmental considerations and rising fertilizers costs, beneficially managing nutrient cycling for sustainable intensification of production is a key factor for modern agroecosystems. Current crop production managements mostly perform removal of nutrients than input, which likely to cause soil nutrient deficiencies in a quite short period. The long-term sustainability of agroecosystems rely on ongoing inputs of NPK fertilizer as well as carbon

management. Soil fertility is an indicator of the soil ability to sustain sufficient crop cultivation in the long-term, and it can be defined by soil physical, chemical and biological activity based on quality of soil organic matter. The soil and plant management are major agent of regulating organic carbon sequestration in agricultural soils. The inorganic fertilizers are the common amendment applied in agricultural practice to improve soil quality and crop productivity and many studies have demonstrated that balanced application of mineral fertilizers can enhance soil organic carbon (SOC) (Liu *et al.* 2014; Powlson *et al.* 2012). However, some research showed that fertilizer could negatively affect SOC and decrease soil quality overtime (Poeplau *et al.* 2016; Fisk *et al.* 2015). Mineral fertilizers, representing form of easily accessible nutrients, may affect SOM decomposition by altering microbial activities (Kumar *et al.*, 2018).

Theoretically, SOC increases when carbon input surplus the decomposition of SOC in agro-ecosystems (Stewart *et al.* 2007). Although, a common humification spectrum of carbon turnover chemistry is well established for diverse soil under different climate conditions, the carbon degradation speed can be significantly affected by variety of agricultural practice. Under specific local land-management conditions, functioning of SOC chemistry, mean residence time and lability could not follow the common trends, and carbon management practice may result in negative outcomes. The cause of the impermanence in the rate of soil carbon accumulation could be due to contrast in the climatic conditions and in local edaphic conditions, crop rotation systems and its crop yield, resulting in further variance in the amount of plant residues returned to the soil (Rengel, 2007).

An increase in the atmospheric concentration of carbon dioxide (CO₂) from 280 mg L⁻¹ (parts per million (ppm)) in the pre-industrial era to 407.25 mg L⁻¹ in 2017, a level not exceeded during the past 800.000 years (Baldocchi *et al.* 2016). This may accentuate radiative forcing and alter the Earth's mean temperature and precipitation (IPCC, 2007; UNFCCC, 2015; FAO, 2017). Rising atmospheric CO₂ may alter the plants C allocations to roots, which will stimulate roots grow and

enhance exudate secretions. This alteration will increase nutrients demand by plants. Moreover, plants exudate will have impact on microbial community which in its turn may have more CO₂ respired by decomposing available soil nutrient. It is now an accepted fact, that the agricultural common practice is taking responsibility for part of current rapid depletion of soil organic carbon. Organic carbon *sequestration* in soils is a potential way for reducing emissions of greenhouse gas (GHG) into atmosphere. The assistance of the agricultural sector is having the capacity to mitigate climate change aftermath should be recognized as a theoretical and practical opinion.

Organic carbon sequestration is the mechanism by which carbon is fixed via photosynthesis and/or following organic residues and preserved in the soil. Considering the CO₂ and SOC sequestration includes three stages: (i) the removal of CO₂ from the atmosphere via plant photosynthesis; (ii) assimilation and allocation into biomass; and (iii) the relocation of carbon from plant to the soil as residue or exudates, where carbon is temporary stored in the form of SOC in the most labile pool, with changeable chemical composition and ability of degradation (Kell *et al.* 2012). The organic carbon stocks evaluation has a particular importance in the following cases: estimation of general reserves of many nutrients in the first place such as carbon, nitrogen, phosphorus and some micronutrients. An understanding of soil organic carbon (SOC) dynamics affected by farming practices is essential for maintaining soil productivity and mitigating climate fluctuations.

Presently, both theoretically and approachable practically land management practice available to sequester soil carbon include efficient use of organic amendments and biofertilization, no tillage, set-aside, cover crop, improved rotations and perennial crops, deep rooting crops, drop irrigation (Smith *et al.* 2001, 2007). Changes in soil C stock tendency due to agricultural practices now recognized as important part of local policy of climate change mitigation and enhancing soil fertility through SOC sequestration.

Since SOC affected by several soil management systems, it is important to know the role of fertilizer such as phosphorus on SOC sequestration. Phosphorus is the second largest fertilizer after nitrogen used globally. A little is known about the effects of long-term P application on SOC pool and sequestration. Since organic carbon degradation is high in Vertisols soil under the semiarid Mediterranean region condition, C stabilization and sequestration is very important for sustainable fertilizer. There are few works on the impact of P application on SOC storage and aggregation under long-term field experiments (Ortas and Lal, 2012). Lopez-Bellido et al. (2010) indicated that long-term soil management experiments are needed to assess soil C sink capacity through a complete life cycle analysis including direct and hidden C costs. Therefore it is very important to search the phosphorous fertilizers apply on carbon and nitrogen sequestration, and the related with soil properties under wheat (*Triticum aestivum* L.) –maize (*Zea mays* L.) sequences cropping system in a Vertisol in semi-arid Mediterranean conditions.

Since 1998, several long-term agricultural researches with annual and perennial cropping systems provide significant insight into the carbon sequestration potential of agriculture in the Ç.Ü. Research Farm located in southeast Mediterranean part of Turkey. Since fertilizer have influence on biomass production and accordingly have effect on soil organic carbon it is important to search the effect of P fertilizer on carbon fixation and flux under long-term field conditions. The study is based on the hypothesis that long-term P-fertilization can affect Carbon Sequestration is soil and plant biomass and impacts soil CO₂ flux. **The objectives** of present study were to: (i) analyze the effect of long-term inorganic chemical P fertilization on the plant biomass carbon uptake and SOC pool for wider environmental protection, (ii) estimate effect of long-term P fertilization on CO₂ flux related with SOC accumulation.

2. LITERATURE REVIEW

2.1. Terrestrial Carbon Sequestration

Soil organic matter (SOM) plays vital role in plant nourishment; maintenance soil functions and release a GHG (CO₂, methane, nitrous oxide) gases in the atmosphere. The combination of particular soil type and its location, vegetation and crop cycle, land-use history and other management practices affects rates of SOC accumulation (Sartori *et al.* 2006). Presently, it is an established fact that soil organic carbon (SOC) and aggregation are fundamental component in relation to the mitigation of carbon dioxide (CO₂) emissions into the atmosphere, which contributes and influence the global climate (FAO, 2017). The role of SOM and greenhouse gases start to be discussed since 1980's when it was emphasized that the relation between soils and climate change and that it was realized that soils played a key role as sink and source of greenhouse gases (Bouwman, 1996; Soussana *et al.* 2004; Rockström *et al.* 2009). Since global warming and climate change is directly related with carbon dioxide which release from surface to atmosphere the area of research on soil C has been greatly expanding for last 30 years.

Soil is the largest terrestrial organic carbon pool (Lal, 2007). Nowadays many research started focusing on C sequestration, motivated by the soil capacity to become a sink for atmospheric carbon dioxide and thus to achieve mitigation of climate change impact and associated with this “win-win” situation improving soil health (McBratney *et al.* 2014). The residence time of carbon in the soil organic matter is variable and depends on current carbon stocks and dynamics of carbon mineralization, through which organic carbon is returned to the atmosphere (Arrouays *et al.* 2014).

The total amount of C in the terrestrial biosphere is 2767 gigatons (Gt) C, and the C fractions in plant, litter and soil organic matter are 19%, 4% and 77% respectively (Wang *et al.* 2010). Measured to 3-m soil depth, including the large C

pool in permafrost soils, total soil C pool is estimated at 4.000 Pg (Lal, 2014) (1 Pg = 10^{15} g). Measured to 2-m soil depth the total SOC pool, to a is estimated to be 2.400 Gt C, that is three times more than atmospheric CO₂ (roughly 800 Pg C) and about five times greater than depository in terrestrial biota C (IPCC, 2007; FAO, 2017). Soils in relatively humid conditions, calculated to have around 120 - 312 Gt SOC, which is 3 times more than the soils under arid conditions (Hillel and Rosenzweig, 2009; Köchy *et al.* 2015). Theoretically, the soil's C sequestration potential refers to the capacity of soil to sequester C as soil organic and inorganic carbon (SOC and SIC) sourced from the atmosphere in the long-term to mitigate climate change. Soil organic C (SOC) pool estimated at 1 550 Pg (in the surface 0–1 m depth) and soil inorganic C (SIC) pool at 950 Pg (Lal, 2008). The SOC pool includes active humus substances and relatively inert charcoal C. Grasslands cover around 70 percent of the global agricultural area, and represent near 20 percent of the world's SOC stocks (FAO, 2017).

Thus, in close perspective SOC stock is a proportion of carbon input and output, and intensify SOC sequestration demand laborious balances between new carbon residue input without an mineralization rate enhancing in order to not cause priming effect and disturbance "older" soil organic matter (van Kessel *et al.* 2000). Carbon-sequestration may occur simultaneously or phase-by-phase with input of labile decomposable compounds and with priming effects (Zhang *et al.* 2017, Sanderman *et al.* 2017).

However, CO₂ absorbed by the land ecosystem, its location and arrangement and allocation still display the greatest uncertainties (Hou, 2013). The terrestrial and atmospheric C pools strongly interact with one another through photosynthesis and respiration (Lal ,2010). The balance of the SOC pools continue to be under pressure of human impacts as an any agricultural practice, deforestation and other land use changes (Hillel, 2009). Lal (2004a) suggested that the carbon sequestration potential of agricultural soils for the near future (25–50 years) would be modest (50–100 Pg C), but important as it is a functional reservoir for CO₂.

Huge efforts from scientists all of the world have been focused on the evaluation of soil organic carbon (SOC) sequestration, interacting and relating to greenhouse gases (GHGs). According to Lal (2014), CO₂ sequestration, represents natural occurring processes of photosynthesis and transition of photosynthetic CO₂ into undefined stable carbons forms (i.e., biochar, humus) and sequestration of CO₂ in biosphere is possible by two ways: terrestrial sequestration and oceanic sequestration.

For agricultural sector, possible way to manage carbon sequestration is the soil management and through crop biomass (Lal, 2014). This is a process of taking away CO₂ out of the atmosphere and converting it to biomass (plants) or to soil organic matter (SOM). Plants arrange 40-60% of photosynthetically fixed carbon into roots and discharge root cells, tissues, and a range of exuded organic compounds (Keiluweit *et al.* 2015). In comparison to C storage, which is an indicator of net outcome of carbon changes from various ecosystem compartments, annual C sequestration is an instant reflection of climate changing and site-specific environmental parameters (Rieger *et al.* 2015).

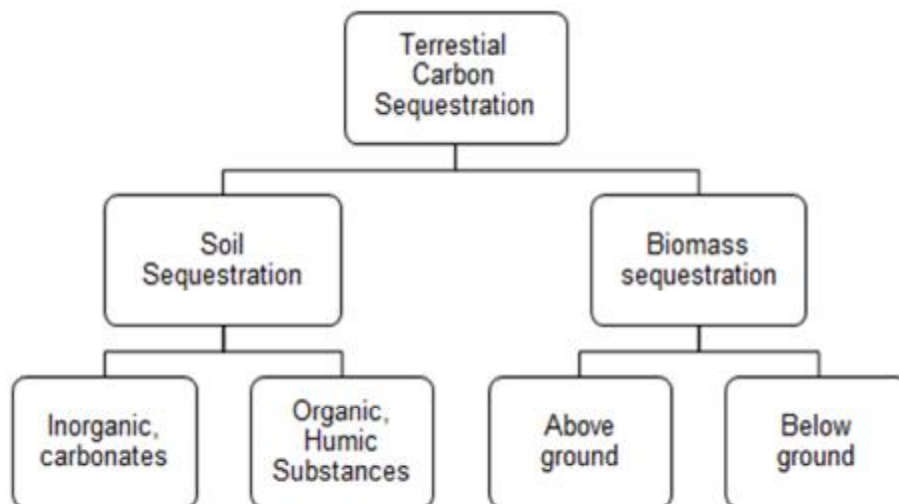


Figure 2.1. Carbon Sequestration (adapted from: Lal 2010-2014)

In the latest soil carbon and soil CO₂ fluxes assessment report, Le Quéré *et al.* (2016) indicated that, since 2006 to 2015, carbon fluxes from land to the atmosphere were higher than the ocean and land altogether can absorb, there with 90% of these fluxes were emitted from fossil fuels and industry sectors.

Different types of carbon influencing the estimation of CO₂ sequestration in soils are presented in Figure 2.2. Theoretically, biogenic carbon is divided into relatively Passive pool (Humic substances) and relatively active pool. Further separation may be as chemical separation (acid/base precipitation) or based on physical characteristics (size of molecules). However, considering humic substances molecular weight, it might be not be clear, due to the fact that humic substances contains various components which have not as yet been properly chemically defined (McDonald, 2004).

Pedologic pool, Total soil carbon	(A) Geogenic, Inactive Coal Carbon 3294 Pg to 3-m depth			
	(B) Soil inorganic carbon 950 Pg to 1-m depth			
	(C) Biogenic, Soil Organic Carbon 1,530 Pg			
	(C1) Slow, Intermediate,	(C2) Stable, passive, recalcitrant (Lignin, Lipids, Fats) Humic fractions		
	DOM/DOC (dissolved organic matter/Carbon, SOM < 0.45 µm in solution) HA FA Humin	Humic Acid (soluble in alkali)	Fulvic acid (soluble in acid)	Humin (insoluble)
		(C3) Labile, active		
		MBC (Soil microbial biomass carbon)	POxC (KMnO ₄ oxidizable C) Plant Litter, Root exudates, microbiota, Polysaccharides	
	POM (0.053 mm and 2 mm in size) partially decomposed detritus		Hot water extractable C, easily available for microbial utilisation	

Figure 2.2. Terrestrial carbon pool

(adapted from: Sarmiento and Gruber, 2002; Lal 2004, 2014; Das R. et al. 2016; M. von Lutzow et al. 2007.; McDonald et al. 2004).

Terrestrial sequestration includes formation of secondary carbonates (Lal 2004). The SIC pool comprises elemental C and carbonate minerals such as calcite, gypsum and dolomite, primary and secondary carbonates. There are primary carbonates may remain in soil while weathering and usually the term "residual-carbonate" is used in the name of such soils. There could be considered further successful practice for carbon sequestration as inorganic carbon. It requires a manipulation of Ca from silicates, water, alkaline pH, and CO₂ (i.e. root respiration). In addition, because soil fungi and bacteria are agents of calcite biomineralization, they might also be manipulated to enhance inorganic sequestration (Monger, 2014). Relatively low rate of SIC sequestration as pedogenic carbonates occurs in arid and semi-arid territories, as well as in agroecosystems with proper irrigation (Lal, 2010).

Concerning over recalcitrant charcoal forms of C are found over a large pedological area, but recognized as a minor part of the total organic carbon. This conditionally inert material reported to be 0-60% of the total C in soils and is in most cases uncorrelated with SOM contents (Lal *et al.* 2001, Baldock, 2007). Charcoal is eliminated from acid-base-wet oxidation of SOM estimation methods, but contained as SOM part in loss on ignition and combustion methods (Weil, 2004). Conceptually, carbon considered as sequestered when it remains in the soil for an indefinitely long time. Marschner *et al.* (2008) speculated that C remains stable because it is transformed from its plant and microbial forms into recalcitrant forms because of their molecular structures.

An additional possible way for terrestrial C sequestration may be achieved through SOM stabilization by physical occlusion within aggregates, chemical interactions with clay minerals, and biochemical recalcitrance (Blanco-Canqui and Lal, 2004; Yost *et al.* 2014; Torri *et al.* 2014). The bonds between the surface of clay particles and organic matter in soils mean that clayey soils can have larger ability of carbon sequestration. Structural aggregates are created and stabilized through the formation of organo-mineral complexes, especially those involving

complexation of organic/humic substances with clay colloids and cations (Ca^{2+} , Mg^{2+} , Fe^{3+} , Al^{3+}) (Lal, 2013). Churchman *et al.* (2014) demonstrated the addition of fine-textured wastes from bauxite mining to sandy soils for 29 years has shown significant increases in SOC that are strongly correlated with the clay contents of the soils.

Soil quality and its assessment are specific and unique and depend on a multiplicity of factors, such as inherent soil characteristics, environmental condition, climate, anthropogenic impact and intended land use, history of management, and environmental protection goals.

Determination of SOC storage dynamics in modern day are focusing on several different techniques, which can be placed into three major approaches (Ellert *et al.* 2001):

1. Flux determination: indirectly, by multitudes estimations of carbon dioxide emission exchanging between soil and the atmosphere.
2. Repeat carbon inventory techniques: directly laboratory measurements, regularly repeated.
3. Assessments of carbon fractions changing: indirectly, by determination of changes of labile and stable soil carbon fractions to predict further carbon dynamics.

Farming process and plant cultivation based on low external input may significantly interfere OM content of soil by exposing fresh plowed topsoil to oxidation process. Thereby, soil organic compounds are released into the atmosphere consequently of their biotic and abiotic degradation and concomitantly, decreasing in aggregate stability due to breakdown and mineralization of binding humic materials.

The balances of carbon added to the systems via photosynthesis (CO_2) and the carbon losses during soil organic matter (SOM) mineralization as well as soil C

removed from cropland by erosion (Bortolon *et al.* 2014) is also affected by soil use and management. Carbon sequestration by soil-plant system recognized as the mechanism of potential of the current greenhouse gas mitigation strategies on global and regional scales (Smith *et al.* 2007).

The Mediterranean region is one of the most densely inhabited areas of the world and the ecosystems have been exploited from the time of ancient cultures to the present. Strategies for sustainable soil management in Mediterranean regions include conserving soil organic matter, minimizing erosion, enhancing soil fertility, and balancing production with environmental sustainability, especially in areas of drought (Mermut, 2014).

In semi-arid lands agricultural practices have higher impacts on decrease of SOM content than under humid climates (Piccolo, 1996). Mainly, soils of the semi-arid Mediterranean basin have high clay and carbonate contents, low water infiltration rates and low soil organic carbon concentration (Ortas *et al.* 2016). Supraoptimal temperatures, high content in calcium carbonate and clay contents with historically excessive tillage stress decomposition of SOM and decrease soil fertility, especially in the southeastern part of the Mediterranean regions. Depletion of SOC pool and the poor soil structure are also exacerbated by continuous cropping on arable land because of the traditional practices of burning crop residues and excessive tillage. Reduced SOM content is one of the major reasons of low crop yields in the Mediterranean region (Ortas and Lal, 2012).

Vertisols are soils that have high content of clays such as montmorillonite mainly substitution in octahedral layer (Bolt, 1978) with continuous a series of shrink-swell process which depends on the moisture and drought. They occupy about 2.4% of the land surface and most of the vertisols occur in semiarid or arid environments; for the smectites to form and persist in the soils, one of the requirements is that the soil solution has a high pH, which results in high amounts of bases (Chesworth, 2008). Vertisols might be found in tropical, subtropical, semi-arid, sub-humid and humid climates with an alternation of distinct wet and dry

seasons, including Mediterranean climatic conditions. Vertisols originates on sediments or weathered rocks with a high proportion of swelling clays. They are typically found in lower landscape positions, dry lake bottoms, river basins, river terraces, and other lowlands those are periodically wet. Vertisols have a good chemical fertility, they have considerable agricultural potential, from rain-fed up to irrigated crops, but adapted water management and fertilization are requirements for constant production (Previtali, 2014). Carbon dynamics is very important in arid and semiarid soils exposed to long-term traditional cultivation practices (García-Orenes *et al.* 2016).

Altın and Barak (2016) in climatic variation research indicated that eastern Mediterranean part of Turkey has thermal conditions getting hotter and increasing daytime temperatures during warm period of the year. Turksever *et al.* (2016) supported that Mediterranean climate become warmer and drier in the last 60 years. And it is speculated on the base of climate change model in the next coming 50 years the region will be under arid climate (Turksever *et al.* 2016).

According to Sakin *et al.* (2010) carbon stock of Turkey is 6.30 - 6.90 Pg C in 1 m soil profile and particularly in Southeast Anatolia Region 0.63 Pg C is found in 1 m soil . Vertisols in Adana region have approximately SOC 7.07 kg C m⁻² and SIC 36.00 kg C m⁻² in 0-100 cm soil depth. The research based on 29 years of long-term fertilization in Eastern China indicated that Vertisol has a large potential to sequester SOC with a high efficiency, while applying cattle manure or wheat straw (Hua *et al.* 2014).

2.2. Biogenic Soil Carbon

Soil organic carbon (SOC) depletion and carbon emissions in agricultural production systems are prevailing through unsuitable outdated farming management practices. The SOC sequestration is a potential negative feedback for GHG emission in agriculture. The agricultural sector has the potential to sequester carbon emissions in soil and the new concept of sustainable

intensification (SI) has been defined as a process where in “*yields are increased without adverse environmental impact and without the cultivation of more land*”. Furthermore, it includes optimizing the agricultural system on natural processes such as nutrient cycling, and simultaneously alleviates negative impacts on natural resources (Arrouays *et al.* 2014). Soil organic matter content and its balancing are decisive to the agricultural management and soil organic carbon is a main indicator of soils condition and its deterioration level. On a global average, management practices that increase dynamic C pools include residue retention, manure, nitrogen fertilization, cover crops, and conservation tillage (Lal, 2005); however, SOC fluxes within a system are contingent upon interacting factors of climate, frequency and cropping sequence and soil biota.

Soil organic carbon sequestration is challenging to consider it under single comprehensive analytical framework (Manzoni and Porporato, 2009; Stockmann *et al.* 2013). The SOM has become a progressively important consideration of many sustainability research areas and SOM turnover and stabilization are recognized as important processes influencing the global carbon cycle (Tatzber, 2009a). Despite the fact that soil scientists are still speculating about the nature and composition of organic matter (Lehmann, 2015; Piccolo, 2016), the recognition of soil organic matter existence and its critical role in issues related to ecosystem and agronomic sustainability, estimating the effects of soil management on chemical characteristics of soil organic matter pools is necessary to maintain soil quality .

A significant distinctive attribute of soil organic carbon storage is the quality and stability of organic matter. The chemical quality of soil organic matter should be considered as an important feature of SOC sequestration strategies. Considerably, it was observed that SOM accumulation and decomposition have a close connection to the molecular characteristics of the organic input to soil (Piccolo, 2012). These characteristics alter the amount of OM accumulated in soil and its chemical reactivity that sustains the C sequestration in soils and its potential

to functioning as carbon pools, source or sink of atmospheric CO₂ (Webster *et al.* 2000).

The biogenic carbon can be theoretically subdivided into three fractions based on its biochemical stability and turnover rates: (i) active or labile C, (ii) recalcitrant or stable C, and (iii) poorly defined intermediate or slow carbon (von Lutzow *et al.* 2007). Parton *et al.* (1987) presented model of five organic carbon pools as a plant residue structural pool (1-5 yr) and a plant residue metabolic pool (0.1-1 yr), an "active" fraction of microbial products and rapidly cycling soil organic matter (SOM) (1-5 yr turnover time), a slowly cycling SOM pool (25 yr), a passive pool (200-1500 yr). The Rothamsted Carbon Model (RothC) (Coleman and Jenkinson, 1996) estimates organic material input as a decomposable and resistant plant material, considering the ratios of the plant material, such as quick-decomposable and slow decomposable plants residue. The century model is suitable to apply in an agro-ecosystem model, where dynamics of several different SOM "pools" compared in that degree of physical-chemical stabilization, under diverse agronomic management and practices and climatic conditions (Kwon *et al.* 2017; Smith *et al.* 2001). This model suggested C:N ratio as a factor of possible sequestration. The microbial community requires carbon supply into soil and consume depending on the quality of the SOM. Similarly to nitrogen availability, C:N ratio assumes of range of SOM decomposition. These three carbon fraction in the mineral soil, from the labile to the most recalcitrant to decomposition also used to be called 'fast', 'slow' and 'passive' in world-used CENTURY model and 'microbial biomass', 'humified organic matter' and 'inert' in RothC model (Davidson and Janssens, 2006). Estimation of various origin carbon-humic substances could play significant role in evaluation of stability and lability of carbon sock in the environment.

Labile C consists of easily decomposable plant litter (such as cellulose and xylan) and root exudates (such as monosaccharides and amino acids), soil

microorganisms, polysaccharides, cellulose and hemi-celluloses with fast turnover rates (Fontaine *et al.* 2007). Stable C fraction consists of lignin, lipid polymers, suberins, resins, fats, waxes other high molecular condensed soil organic matter (SOM) and non-hydrolysable SOM with half-life varying from years to decades (Stevenson, 1994; Chen *et al.* 2009). This fraction also contains humified products formed by biological transformation. Humic fraction can be further divided into humic acids, fulvic acids and humin, which are most resistant to decomposition. Inter-mediate C fraction has turnover rates from decades to centuries and consists of charcoal, pyrolysed and black C (Chan *et al.* 2009).

Slow or intermediate SOM may be described as partially decomposed residues and decay products, such as amino compounds, glycoproteins and aggregate protected POM (Wander, 2004). Inert SOM described as a most non-reactive organic matter, that affects the physical properties of the soil. It has a potentially low sorption capacity. This pool is physico-chemically protected against decomposition and its half-life is between decades and centuries (Strosser, 2010). Clemmensen *et al.* (2013) identified root-associated fungi as important regulators of ecosystem carbon dynamics AMF-derived labile carbon (glomalin) may lead to the formation of more stable aggregates, protecting organic matter (Miller and Jastrow, 2000) and thereby preserving SOC pool (Grant *et al.* 2005).

To sum up, humus is organic substances, which has lost contact with the cell and tissue structures of plant residues, although some part of humic substances and may be still within the dead plant tissues.

No simple and applicable method was accepted to fractionate all of these soil carbon forms. Several methods for measuring soil carbon stocks have been promoted in recent years as a way of dealing with current soil C data crisis, these techniques such as dry combustion, wet combustion and loss-on-ignition.

2.2.1. Passive Soil Organic Matter and Humic Substances

The humification is a global phenomenon, and humic substances of all soils have a common structural principle. The SOC dynamics have been characterized by the “Humus Concept” where composition is supposed to be to some extent controlled by molecular structure of carbon input. Different chemical extractions result in unextractable residues with complex polymeric macrostructures that are considered to be inherently chemically stable (humins and humic acids) (Hartemink, 2014).

Humic substances (HS) are the part of soil organic matter and occur to be complex and diverse in character organic compounds, HS are the most ever-present natural non-living organic compounds found in all terrestrial and aquatic environments. Some of soil qualities depend on humic substances nature, such as indicator of soil fertility, contribute to soil pH level, nutrient availability, weathering and leaching rate (Nardi, 2009). Some studies estimated the biological effects of humic substances and showed enhanced plant growth when plants were exposed to humic matter (Rose *et al.* 2014). Furthermore, it was shown that HS have impact on the soil physical properties, soil aggregate formation and attribution to sustaining plant growth. The molecular characteristics for many features of humic substances still needed to be investigated.

A number of different studies have shown that HS are very complex and heterogeneous molecular systems. Although the centenary history study of humus, the generalized terms humic acids (HA), fulvic acids (FA), and humins cover the major fractions still used to describe HS components, but the boundaries between these fractions have not been yet clarified in chemical terms (Piccolo, 2002). Aliphatic, carboxylic and aromatic groups prevail in unlike proportions; hence HS has different chemical properties. Depend on solubility in acidic or alkaline extractant HS are consequently sorted into three fractions: fulvic acids (FA), humic acids (HA), and humin (Stevenson, 1994). Extracted with strong base humic substances subsequently acidified, then the products resulting as: (I) a

nonextractable residue called humin; (II) precipitates with acidification material is called humic acid; and (III) remaining in the acidified solution material is called fulvic acid. Should be noted, these terms do not refer to single certain biochemical compounds but to a variation of compounds with altogether has a similar origin with wide range of common characteristics (Manahan, 2011).

Stable humic substances redox properties could behave as electron acceptors or donors in soil thus can affect the oxidation of metal ions and their mobility (Aeschbacher *et al.* 2009; Karabcová *et al.* 2015). The possible impact on the HS stability determined by many circumstance such as water content, pH, concentration and spatial distribution of functional groups in HS matrix, and ionic strength of soil solution (Wilson *et al.* 2008; Tunega *et al.* 2014)

Tillage practices have different effects on distribution of fractions, mostly depending on the purpose of research. A long-term field study by Szajdak *et al.* (2003) reported that humic acid-bound amino acids were higher in no-tillage soils compared with conventional tillage practice, while fulvic acid-bound amino acids were higher in conventional tillage practice. The positive HS effects on yield such as stimulating plant growth could attribute to HS phytohormone-like activity (auxin-like) (Nardi *et al.* 2016).

2.2.2. Labile Organic Matter

Not all of the compounds, which have passed humification stage, will be stored in the soil as humus substances. Some fractions of soil organic carbon have rapid degradation rate and short residence times in the soil (Haynes, 2005). The nature of linear or cyclic carbon skeleton, the presence of functional groups and its existence in soil depends on a large number of factors.

Aliphatic, cyclic, and aromatic organic acids have significant part in soil and rhizosphere ecology, as well as in organic matter decontamination. The SOM degradation and carbon mineralization may be interpreted as by changes in its group chemistry (e.g. aliphatic, aromatic, polysaccharides) such as the increasing in

aromatic to aliphatic groups, meanwhile stability may be characterized by prevalence of aromatic groups (Hsu and Lo, 1999). The dynamics of SOM decomposition (mineralization) rely on two conditions: (i) soil moisture, temperature, porosity and pH (ii) the features of the C compounds and their availability for microorganisms (Capriel, 1997). The multistage SOM degradation process determined by the combined response of extra- and intracellular, enzymatically mediated reactions to temperature (Blagodatskaya, 2016). Enzymes secreted by plants and microbiotas are the primary contributor in organic matter decomposition (Nannipieri and Eldor, 2009). The temperature sensitivity of decomposition rate increases with increasing molecular complexity of the substrate (Davidson and Janssens, 2006). In this regard, notion sustainability of organic compounds in soils is much more complicated because it based not only on the structure of molecules, but also takes into account environmental situation. Half or even the greater part of newly formed humic substances quickly involved in biological processes - this is used to be called "labile" part of humus or labile carbon fractions. Biederbeck *et al.* (1994) described Labile C forms as a readily decomposable organic matter with temporal fluctuations and labile organic matter considered as fragile carbon-fraction (Baldock and Nelson, 2000). Although the work done have shown that low-molecular compounds, especially saccharides, also create highly stabilized sorption complexes (Schulten and Leinweber, 1999).

Different labile SOC pools, such as dissolved organic carbon, microbial biomass carbon and permanganate-oxidizable carbon (PoxC), have recently received attention due to their sensitivity to agricultural management practices (Culman *et al.* 2012; Lucas and Weil, 2012). Estimation of labile soil organic matter is important because it is considered as not sustainable to mineralization, especially when they are not physically protected in soil aggregates (Six *et al.* 2000; Tatzber *et al.* 2015).

Total organic carbon measurements might not be sensitive indicators of changes in soil quality. Applying of some method that can estimate labile carbon

fraction constituting a favor to be used for a practical purpose for the characterization of the soil organic carbon reacting to different management practices. Loginow *et al.* (1987) introduced methods of chemical fractionation of SOC, based on its state of being labile to the oxidation with KMnO_4 solvent of various molarity, where permanganate was chosen based on the proposition that breakdown of organic matter in the soil by microbiological consumption is related to an enzymatic oxidation process. Hereinafter several new modified methodic and publications brought clarity in the process with applying this method and have determined the lability terminology in the case with SOC fractions and its residence time (Blair *et al.* 1997, 2001). Weil *et al.* (2003) called this carbon fraction as 'Active C', and now it has various names including 'readily oxidizable carbon'(ROC), labile carbon (LC) and 'permanganate oxidizable soil organic carbon' (such as POC; OxidC; PermOx C).

Suárez-Abelenda *et al.* (2014) shows that KMnO_4 may oxidizes lignin, however it almost does not involve some SOM components such as amino acids, structural carbohydrates and sugars and those are recognized as easily assimilated by soil microorganisms. When KMnO_4 used as oxidizing for aromatic aromatic compounds, as output never more complex products are formed. Non-aromatic substances (cellulose) give mainly oxalic acid, CO_2 and water (Yan and Qi, 2014). Benzenepolycarboxylic acids (BOD) are formed only from compounds with a ring that is repeatedly replaced by carbon atoms, and in the presence of oxygen substituents, the benzoic structures split and a significant part of them is unaccounted, moreover Maximov *et al.* (1977) indicates that under the Permanganate action aliphatic and methoxyaromatic structures are destroyed.

It was recently shown that active carbon (AC) fraction was found to be significantly affected by management practices; moreover, AC shows strong dependency to Nitrogen-based and Phosphorus-based manure treatments (Mirsky *et al.* 2008). Meanwhile, some authors concluded that active carbon is a relatively poor indicator of labile C (Tirol-Padre and Ladha, 2004). Notwithstanding,

allowing mild reaction conditions effective heterogeneous oxidizing agent such as KMnO_4 has clearly shown ability to use it as criteria of multistage SOM fractionation procedure in response to agricultural practice. This makes the quantification of AC pool promising soil tests to assess management impact.

2.3. Relationships Between Soil Organic Matter And Phosphorus.

2.3.1. Soil Phosphorous

Protection of soil health and quality under intensive arable land for food production is a major challenge for soil science and agriculture. Like many of the reviews agreed, soil C is a key contributor in sustaining and improving soil quality. Land used for crop cultivation is conventionally managed with inorganic fertilizer in order to maintain high yield crops (Stewart *et al.* 2005). Furthermore, the characteristics and change within a system of the SOM and P association can be a basis for predicting the changes on soil quality (Aguilar *et al.* 2013).

Phosphorus is an element considered as an essential macronutrient for plant nutrition, its availability plays a crucial role for carbon cycling in terrestrial ecosystems. Phosphorous, is provided for pasture development and maintenance through superphosphate fertilizer application (Morton *et al.* 1999). A lack of P supply limits a plant ability to complete its growth and reproduction (During, 1984).

However, with increased policy- makers and public attentions to soil carbon and to issue of regular fertilizer application, there is not much information about as far as the fertilizers may affect soil organic C and its composition (Schipper, *et al.* 2007). Little is known about the effects of long-term P application on SOM and its relationship with carbon flux in Vertisols in the semiarid Mediterranean region. This subject arouses a great interest, in the past years several studies reported an increase in heterotrophic respiration after P addition, mostly in P-limited forest soils (Cleveland and Townsend, 2006; Reed *et al.* 2011; Liu *et al.* 2013).

The total amount of P (plant biomass, litter and soil) excluding occluded P in soil is 17 Gt P in the terrestrial biosphere, 33% of which is stored in the soil organic matter if biochemical P mineralization is modelled, or 31 Gt P with 67% in soil organic matter otherwise (Wang, 2010). In soils, P derives mainly from weathering of the primary mineral apatite (Schlesinger and Bernhardt, 2013), and the average ranges of total P (Pt) varies 200-800 mg kg⁻¹ (Cross and Schlesinger, 1995). Despite the large amounts of total P it is a limiting factor for plant growth because plants uptake only phosphate PO₄³⁻ from the soil solution in the form of orthophosphate and its availability mainly relies on the soil pH, where the lowest content of available P is usually detected in strongly acid and strongly alkaline soil (Marschner, 2007). Some of the other soil features (include clay content, soil pH, CaCO₃ and organic matter) can also have strong impact on phosphorus availability.

Phosphorus belongs to the elements, which may appear with a different (variable) valence, and while the formation of chemical bonds they can act as donors or as acceptors of electrons. Phosphorus usually forms compounds with a valence of 3 and 5; there is in soils phosphorous has tendency to exist in 5-valent compound. In a typical for soil pH range 2-8 predominant particle is H₂PO₄ and at pH from 8 to 11-12 is HPO₄²⁻ particle (Bünemann and Condron, 2007). Some of these phosphates represented in soil solution by CaHPO₄, CaH₂PC₄, FeH₂PC₄⁺. The phosphate ion gets into the soil solution as a result of mineralization organophosphates or by fertilizers. If the concentration is increased, part of phosphate is fixed by solid phases. Chemisorption is performed due to interaction with the phosphate ions with atoms Al, Fe or Ca on surface of solid phase of soil (Schlesinger and Bernhardt, 2013). The same types of reactions are characteristic of the formation of insoluble precipitates, the formation of which depends mainly on the pH and the concentration of ions Fe³⁺, Fe²⁺, Al³⁺ and Ca²⁺ in the soil solution (Sharma *et al.* 2013). Well known, at high pH the concentration of phosphate ions in the solution is controlled by Ca²⁺, and then solid phase phosphates represented by hydroxyapatite Ca₁₀ (PO₄)₆ (OH)₂. The high adsorption capacity towards

phosphate has a calcite CaCO_3 , on its surface firstly during chemisorption are formed amorphous calcium phosphate and then passing into crystalline form (Orlov, 2005). Adsorption and precipitation of insoluble Ca phosphates in calcareous soils are main reasons of the loss of applied P fertilizer (Eriksson *et al.* 2015; Delgado and Torrent, 2000). These features cause high chemical activity of P, the variety of compounds in the soil and play important part in various biochemical and abiotic soil processes.

The forms of inorganic P (Pi) and Organic P (Po) are poorly soluble in the soil solution and considered as not directly available (Lopez-Bucio *et al.* 2002). Some enzymes mineralization of organic P, such as induced by the exudation of phosphatases may control soil P dynamics and availability (Turner and Engelbrecht, 2011). Microbial P immobilization plays role in declining P availability by removing inorganic P from the soil solution, particularly in a presence of accessible soluble carbon for microbial growth (Rabary *et al.* 2008; Olander and Vitousek, 2004). Fertilization with phosphorus (P) consistently suppresses phosphatase activity (Clarholm, 1993).

The most important phosphorous form for agriculture is the plants available orthophosphates P-forms. Agricultural fertilizers such as mineral P fertilizers, animal manure and compost, provide P that is readily available to plants.

2.3.2. SOC and Phosphorous interaction

Soil organic matter has a significant part in adsorption of organic compounds and inorganic cations. Soil organic matter is a source of P from which it can be obtained by plants and soil microorganisms (Kirkby *et al.* 2011), in addition as an energy source and carbon used in soil microbiotas activities such as the exudation of enzymes engaged in phosphorus mineralization (Allison and Vitousek, 2005). SOM content has an important role in relation to the P sorption capacity of soils as the main constituent of the soil sorption complex which is taking part in binding anions in the soil (Debicka *et al.* 2016). Organic matter (OM) interacts

with P in soils in a variety of ways that potentially influence these P sorption reactions. According to Guppy (2005), the mechanisms through which OM can impact P phytoavailability are include metal complexation and dissolution reaction and formation of metal bridges providing increasing phosphorous sorption, as well as OM ability to maintain pH and enhance P availability. On the other hand, some studies have indicated that soil organic C may negatively affect soil P availability because of embedded into organic complex compounds (Huang *et al.* 2013; Schlesinger *et al.* 2013). Soil organic matter can also provide binding sites for phosphatases to protect them from degradation (Tabatabai *et al.* 2002) by complexing and sorption the humic acid includes mineral components, especially iron, aluminum, calcium and phosphates. The remains of the nucleoprotein, inositol phosphates, and phospholipids represent phosphorus in organic compounds. Several studies (He *et al.* 2011; Barančíková *et al.* 2007; Makarov, 1996) indicated P forms in humic fractions were predominantly organic. The association between phosphate and humic substances is probably through polyvalent metal bridging cations and it is *not classified* as organic phosphorus (Turner *et al.* 2005) but relate to these organ-mineral forms of soil P (Gerke , 1992). Nissenbaum (1979) showed that Phosphorus presents in humic fraction, thus apparently might affect P cycling and plant nutrition. Erro *et al.* (2012) indicated that humic acids enhance P_i solubility and inhibit P immobilization. Whatsoever, more data are required to provide information of interaction between phosphorous fertilization and humic-bond phosphorous to link impact on C sequestration in agricultural ecosystems. Presently, there is a growing interest of the soil scientific community on the fraction and lability of Phosphorus-humic fractions interaction, considering the crucial role of humic acids in P cycling and plant nutrition (Makarov, 2005; He *et al.* 2009a).

Humic substances in interaction with phosphorus in the soil can decrease the phosphorus fixation and increase the phosphorus uptake of plants (Hua *et al.* 2008). Grossl and Inskeep (1991) reported that octacalcium phosphate (OCP) is

an important reaction product of P fertilizer addition to calcareous soils and as a prior solid phase in the formation of thermodynamically more stable hydroxyapatite (HAP), in their study OCP precipitation was inhibited by Humic acid. Moreover, authors indicated that complexation of soluble Ca^{2+} by organic ligands can also reduce precipitation rates by lowering the free Ca^{2+} concentration in solution.

The role of natural organic matter presumably may be associated with the organic molecules (humic substances) with high cation chelating-complexing ability. Baigorri *et al.* (2013 and 2016) suggested that bioavailable micronutrients with metallic character in soil solutions of alkaline and calcareous soils are forming stable complexes with dissolved organic matter and it plays beneficial role and concerns other plant nutrients such as inorganic phosphate (Pi). There is also an evidence of fungal phytase can release humic-bond P into soluble inorganic P (He *et al.* 2009a, 2009b). Baigorri *et al.* (2013) in a study of general synthesis procedure for phosphate-metal-humate-based fertilizers, has provided way of creation of humic complex. The theoretically possible way of phosphate units of $\text{Ca}(\text{H}_2\text{PO}_4)_2$ of mineral fertilizers fixated by HS should follow further reaction: $\text{Ca}(\text{H}_2\text{PO}_4)_2 + \text{HS} \rightarrow (\text{H}_2\text{PO}_4)_2\text{-Ca-HS}$.

2.3.3. Phosphorous Fertilization and its Impact on SOC

Influence of mineral fertilization on humus state of soils is interesting not only from an agronomical point, but also because it let us to follow the processes of changing the salt-ion composition of soil.

Improvement in soil carbon pool with fertilization might be caused by several factors such as large biomass production resulting in more plant residue return to soil, and long-term arranged in right proportions fertilizer application can reduce carbon mineralization by soil microbiota. Application of organic amendments such as manure, compost and biochar is one of principle key to SOC sequestration strategy. Organic fertilization management from both agronomic and

environmental perspectives has been received a lot of attention in soil science community and has been shown to be a perspective practice for soil aggregate formation rate, SOC stability and has positive effect on soil microbial activity. Numerous of long-term experiments have demonstrated that the SOC sequestration rate significantly bigger under organic manure application than with mineral fertilizers. When assimilable resources are able to be obtained, microorganisms may decrease the production of enzymes that degrade complex substrates (Allison and Vitousek, 2005). Kuzyakov (2010) highlighted that *Priming effect* is defined as a short-term change in the turnover of soil organic matter caused by treatments as organic C applying to the soil and accelerated CO₂ evolution in response to the activation of microbial metabolism; higher microbial biomass turnover is not related to the SOM turnover and is termed as apparent priming effect (APE). Basically, the addition of easily decomposable C compounds to soil may concomitantly induce an acceleration of mineralization processes and enhance the tendency in losing SOC pool (Kuzyakov *et al.* 2006, Luo *et al.* 2014).

The quantity of no-tillage SOC storage is not well predictable due to large dimensional, geochemical variability and difference in land-use management. Ashworth *et al.* (2014) suggests that sub-soil C pools demanding more long term (more than 8 years) of cover crop and balanced residue additions combined with no-till management for greater C conservation. Numerous studies have found negative impact on total SOC caused by cover crop production change and priming effect when labile carbon stimulating metabolism of stable C (Fontaine *et al.* 2003, Wu *et al.* 1993). These findings are supported by Sanford *et al.* (2012) who indicated that within 20 years long term experiment, SOC was decreasing, despite the no-tillage, perennials crops and manure usage, which are considered as the agricultural best management practices. However, Ohno *et al.* (2009) results indicate that the HA and FA fractions were not significantly affected by long-term tillage and cropping. Karabcová *et al.* (2015) indicated that organic fertilizers can influenced humic acid, fulvic acid but not some of labile carbon forms.

Nevertheless, it is well known that P-fertilization can affect carbon storage capacity of soil and CO₂ fluxes, by several mechanisms: (a) increase metabolic process and respiration, (b) reduce the abundance of arbuscular mycorrhizal fungi and (c) decrease crop root growth (Ericsson, 1995; Marschner *et al.* 1996), which is leading to lower root-derived carbon input.

Recently, Poeplau *et al.* (2016) reported a significant net decrease in SOC stocks after long-term P+K but no -N fertilization compared with unfertilized control soils in Swedish agricultural long-term experiments, although plant net primary production was positively affected by PK fertilization. Meanwhile, Hartman and Richardson (2013) hypothesized that N limits microbial biomass synthesis, while P limits microbiotas metabolism. Moreover, Fisk *et al.* (2015) indicates that P addition, in the form of fertilizer can lead to SOC losses in any ecosystem and not only in P-limited soils. This can be explained by role of phosphorous to be an essential element for the production of enzymes, which can have impact on breaking down organic matter. Increases in soil organic C content can provide more energy and C-structure materials for microbes, leading to elevated production of phosphatase enzymes involved in the mineralisation of soil organic P (Allison and Vitousek, 2005). Meanwhile, Iovieno *et al.* (2009) reported that in Mediterranean area the increasing of microbial activities induced by compost amendments was in accordance with an increase in crop yield only for the soil with the low SOC content. Mori *et al.* (2016) reported that P-fertilization increases the dissolved organic carbon (DOC) contents along with CO₂ emissions and suggested that P addition had changed soil microbial activities, but stimulated soil respiration by P addition is not obligatory co-occur with increased microbial biomass carbon. The long-term effect of phosphate fertilizers application on microbial activities was shown have inhibition effect of substrate-induced respiration by streptomycin sulfate (fungal activity) and cycloheximide (bacterial activity) and microbial biomass carbon (Bolan *et al.* 1996). Chandini and Dennis

(2002) has demonstrated a significant decrease in microbial respiration and metabolic quotient (qCO_2) after phosphorous application.

As mentioned above and well published, phosphorous fertilizers can negatively affect microorganisms such as arbuscular mycorrhizal fungi (AMF), thus leading to a negative effect on SOC. However, the priming effects of mycorrhizae on soil carbon balance also occur and thus aggravate the balance between carbon stabilization and soil carbon priming effects (Brzostek *et al.* 2015).

Benbi and Brar (1994) suggested that wheat response to fertilizer P is related to the combined effect of available P and soil OC content and the reliability of fertilizer recommendations based on Olsen P might be improved on some alkaline soils under consideration of SOC content. However, as mentioned above some studies postulated that an accumulation of soil organic matter might decrease soil P availability due to the inclusion of P in organic form (Huang *et al.* 2013).

Hou *et al.* (2014) observed that content of soil organic P increased significantly with increasing soil organic C, whereas the percentage of soil total phosphorous (P) had tendency to increase with soil organic C only when SOC was low, but was relatively stable when SOC was high. Moreover, soil organic C was highly correlated with P sorption index. Authors suggested that accumulation of organic C might increase, rather than decrease, under the availability of phosphorus condition. Wang *et al.* (2006) found that soil organic C concentration was positively related to the concentrations of soil soluble organic-P fractions, whereas it was negatively related to the concentrations of soil soluble inorganic-P fractions. Souza *et al.* (2016) indicated that eleven years P fertilization did not change the total organic carbon, but promoted the accumulation of permanganate oxidizable carbon. Reversly, Ortas and Lal (2012) shown that since soil P level increased straw biomass and consequently SOC increased.

Sartori *et al.* (2006) reported that annual fertilizer treatments had no significant change in soil C pool in the long-term experiment under pine plantation, despite the generally observed increases in soil available N and P. Van Cleve and

Moore (1978) found strongly increasing soil respiration with N and P fertilization, but slightly decreasing soil respiration with K fertilization.

Many studies have suggested that inorganic and organic fertilization, including straw return, greatly increase SOC stocks (Bhattacharyya *et al.* 2010; Ghosh *et al.* 2012; Powlson *et al.* 2011; Yan and Gong, 2010), and a combination of organic and inorganic fertilization results in a greater increase in the carbon pool than chemical fertilization alone (Brar *et al.* 2013). Fertilizer induced increases in C inputs do not always translate into greater soil organic matter content. For example, in a 40-yr experiment, application of P fertilizer to a New Zealand pasture increased herbage dry matter production from 4 to 11 t ha⁻¹yr⁻¹, but had no effect on soil C (Stewart and Metherell, 1998).

Zhang *et al.* (2010) had measured SOC dynamics under long-term fertilizations in arable land of northern China with a double-cropping system of corn (*Zea mays L.*) – wheat (*Triticum Aestivum L.*) rotation, concluded that for the arid and semi-arid areas, balanced mineral applications (e.g. NP and NPK) is not sufficient to maintain SOC level and leading to a decreasing trend in SOC and require additional amendment. Other studies have shown that modified cultivation methods such as no-tillage and agro-forestry increased the SOC concentration compared with conventional cultivation practices (Larsen *et al.* 2014; Dimassi *et al.* 2014; Sierra *et al.* 2013).

Research conducted on a clay smectitic vertisoil in Mexico to evaluate the use of traditional-deep and no-tillage systems on SOC dynamics for wheat and corn cropping systems and results from this study indicate no-till on N-fertilized CW systems can potentially increase SOC sequestration on large areas of irrigated Vertisols (Follet, 2005). Meanwhile, Morell Soler (2012) studied the effects of long term adoption of tillage practices, with barley crop and N fertilizer effect on SOC balance in the Mediterranean semi-arid Spain soil and results indicated C outputs were increased under NT, but less affected by N fertilization, and long-term medium and high N fertilization increased the stock of SOC by 3.4 and 4.5 Mg C

ha⁻¹ in contrast to unfertilized plots. The author suggested long-term adoption of conservation tillage practices (no-tillage) together with adequate N fertilizer use, proved to be effective tools to improve sustainability of semi-arid Mediterranean drylands and to store C in the soil.

Investigation on the soil organic C and P fractions relationships is needed to assess complexity of soil organic C accumulation along with soil P availability that plays an important role in further research undergoing on the phosphorous input-limitation and carbon sequestration in terrestrial ecosystems (Wang *et al.* 2010).

2.4. SOC, Phosphorous and Carbon Dioxide Flux

2.4.1. SOC Effects on Carbon Dioxide Fluxes

There is an intense interest in investigation of natural sinks of atmospheric carbon the strong relationship between the atmospheric, pedologic and the biotic C pools which are major components of the global carbon cycle (GCC) and understanding and maintaining these interactions is a strategy to sequester atmospheric carbon dioxide (CO₂) (Lal, 2010). Long-term CO₂ sequestration is required stabilized SOC (Zimmerman, 2012), where SOC sequestered should start from the atmospheric CO₂ pool and passed through plants into the soil humus as root exudates and as plants residue (Olson, 2013; Lal, 2016). According to different climate IPCC scenarios (2013) predicted of increasing of CO₂ levels of ~500 to ~900 ppm by the end of this century. Anthropogenic emissions are presently estimated at ~10 Pg C year⁻¹ from fossil fuel combustion and land use conversion including soil cultivation and drainage of peatlands (Lal, 2014). The net flux of carbon from the lithosphere and the biosphere into the atmosphere is estimated to be about 3.2 Pg C yr⁻¹ (Janzen, 2004). The atmospheric C pool, estimated at about 800 Pg, is increasing at a rate of about 4.2 Pg C/year. In a global perspective, agriculture contributes ~5.0–5.8 Gt CO₂ yr⁻¹ or ~11% of total greenhouse gases emissions, without calculation of land-use changes (Smith *et al.*

2014). Pries *et al.* (2017) reported that in an experiment whole-soil-profile warming up to 4°C more was found that CO₂ production from all soil depths increased with warming and soil respiration increased by 34 to 37%. However, having a significant part in managing climate change, the CO₂ emissions associated with an agricultural practice have not been generally included in C sequestration analyses and formulation.

Meanwhile looking regionally, GHG agricultural emissions are playing serious role at contributing and account an average of 35% of emissions in developing countries and 12% in developed countries according to countries' GHG emissions inventory reports to the UNFCCC (Richards *et al.* 2016). United States Environmental protection agency noted that CO₂ that ecosystems remove from the atmosphere by sequestering carbon in biomass, dead organic matter, and soils, which offset approximately 20% of emissions from this sector. The increasing atmospheric CO₂ concentrations potentially expected to intensify plants growth, thus with increasing plants mineral nutrients demand. Since P is involved in numerous metabolic processes and play role for energy production in photosynthesis, in a condition of increasing CO₂ in atmosphere could cause higher inputs of P fertilizers that could accelerate the depletion of rock P reserves (Jakobsen *et al.* 2016). Jin *et al.* (2015) concluded that increased P demand by plants apparently happens with increasing CO₂ portion in atmosphere, while stimulation of photosynthetic activity and further growth and, moreover, root morphology changes, due to carbon fluxes along the glycolytic pathway and tricarboxylic acid cycle. Root exudates may initiate P mobilization by the P-chelation, thus alter microbial activity in the rhizosphere (Jin *et al.* 2015).

Soil carbon depletion given mostly to CO₂ produced during decomposition of organic matter, and root respiration, as well as carbon leaching and erosion are important components of C balance considering long time scales (Trumbore, 1997). Prevalence and magnitude of local CO₂ source and sinks depend on factors such as biological activity, soil properties and environmental variables.

The CO₂ efflux sources from soils can be categorized to root respiration, respiration of rhizosphere biota, decay of plant residues, the priming effect induced by root exudation or by addition of plant residues, and basal respiration by microbial decomposition of soil organic matter (Kuzyakov, 2006). Plants may play a key role in soil respiration through their root biomass, and it was found soil CO₂ flux increased with root biomass (Luo *et al.* 2014; Melling *et al.* 2014). The SOC sink capacity depends on both natural and the anthropogenic factors. Important among natural factors are soil properties, landscape position, drainage, and climate (Lal, 2014).

The effect of fertilization on the soil C budget has traditionally been assessed by measuring total organic C in the soil. Shifts in soil C accumulation tendency due to agricultural management practices recognized as important part of local policy of climate change mitigation and increased soil fertility through SOC sequestration. The stabilization of soil organic carbon by hydrophobic protection mechanisms improving carbon sequestration by means of decreasing microbial activity, soil organic matter mineralization and CO₂ flux (Atanassova, 2014).

Carbon dioxide (CO₂) is the end product of microbial decomposition of organic material. However, microbial metabolism consists of many concurrent anabolic and catabolic reactions. The multistage decomposition of SOM and ultimate CO₂ release depend on the combined response of extra- and intracellular, enzymatically mediated reactions to temperature (Agren and Wetterstedt, 2007). According to Blagodatskaya *et al.*(2016), the extracellular steps of SOM decomposition include: (1) the release of monomers from polymeric compounds, and (2) the active and passive transport of monomers into microbial cells, where the subsequent intracellular enzymatic reaction chain includes the catabolism of consumed organic substances, releasing CO₂ as the end product. The extra- and intracellular enzymatic steps have great significance in the SOM reaction to global warming, but with different responses and sensitivities to temperature change (Blagodatskaya *et al.* 2016).

Dealing with CO₂ flux from agricultural soils, we are supposing that these emissions are result of root and microbial respiration, however, some research have examined whether this is valid for calcareous soils and alkaline soil. Tamir *et al.* (2011) study showed that the dissolving of soil carbonates could contribute up to 30 % of the CO₂ flux from calcareous soils in Israel. West and McBride (2005) showed that CaCO₃ reacts with acids, (e.g., oxalic acid from exudates) create one mole CO₂ for every mole of CaCO₃, which is approximately 3% of the mean total net CO₂ flux, which can be taking into account only under limestone weathering condition (i.e., high soil water CO₂ concentrations) (Dannenmann *et al.* 2007). It was shown, that parent material had not significant impact on the organic chemistry or carbon dynamics in the top organic horizons of alkaline soils, moreover, alkaline parent material had a larger heterotrophic CO₂-to-SOC ratio, it suggests that the larger SOC stocks in the alkaline soils were not caused by a higher chemical resistance to mineralization (Nambu *et al.* 2008). Ramnarine *et al.* (2012) in calcareous soil an incubation experiment , resulted that the amount of CO₂ emitted was up to 74 % as conventional tillage as no-tillage area. Fraser *et al.* (2016) reported that in calcerous soil major flux was derived from microbial respiration, whilst the remainder was a consequence of enzyme activity and there was no significant contribution to the total CO₂ efflux from an abiotic source such as carbonate dissolution.

2.4.2. Phosphorus Effects on Carbon Dioxide Flux

Negative effects on SOC accumulation have been covered recently and explained as one of priming effect and by stoichiometric decomposition theory where varying reaction of labile and stable C on N and the importance of P insufficiency for decomposition process in some soils will demand restructuring C-cycling where P application may increase SOC decomposition (Craine *et al.* 2007). Curtin *et al.* (2002) result showed that the CO₂ fluxes were slightly

influenced by fertilization treatments. The authors also suggested that mineral fertilizer amendment would be expected to increase CO₂ fluxes due to accelerating the main sources of soil respiration, such as root respiration, decomposition of plants residues and soil organic matter mineralization, however eventually the anticipated increase in rhizosphere respiration due to greater crop growth on well-fertilized soil was not observed. Hassler *et al.* (2015) reported that under different land-use management in tropics, annual CO₂ fluxes were positively correlated with soil organic carbon (C) content and negatively correlated with extractable P. The numerous scientific reports investigating nutrient effects on SOC degradation especially with the center of interest on nitrogen (N) application and the reported impact varied regarding to different practice and soil. In the comparison of variety of soil microbial communities, Cleveland and Liptzin (2007) postulate an average microbial C:N:P molar ratios of 60:7:1, and Van Meeteren *et al.* (2008) on leaf litter microbial communities also reported low C:N:P ratios as 16:4:1. Some hypothesis shows negative relationships between C:P and N:P ratios and growth rate (Sturner and Elser, 2002). Murphy *et al.* (2015) suggested that the SOM of the low productivity soil was intrinsically more stable where the greater SOM mineralization in the high productivity soil supported a larger microbial biomass, also highlighted the significance of SOM heterogeneity as a substrate for microbial biomass and that the bulk SOM C-to-N ratio is an insufficient predictor of N-flux associated with C-mineralization. Moreover, Murphy *et al.* (2015) indicated that SOM mineralization due to priming had a C-to-N ratio of 5, while during basal respiration a C-to-N ratio was 20. Meanwhile, Cleveland *et al.* (2006) indicated that CO₂ emissions were suppressed by N, but accelerated by P addition, when combined with glucose. Generally, a discussion about mining mechanism for P has not sufficiently covered in scientific reports, as well as P impacts on SOC balances and cycle.

Popelau *et al.* (2016) data suggests that Glucose+P addition may foster biosynthesis as compared to Glucose addition alone. This was also observed by

Fisk *et al.* (2015) and showed that mineral phosphorus fertilizer application in agriculture can cause increasing SOC mineralization in any ecosystem and not only in P-insufficient soils.

The carbon pool and its accumulation rate mostly depend on the balance between soil-C inputs/influx (sequestration) and outputs/efflux (mineralization, soil respiration, microbial decomposition, etc.) (Satori *et al.* 2007). Soil carbon pools have a stable nature, while carbon fluxes have a rapid response to environmental changes. However, currently, it cannot be concluded if fluxes, stable and labile pools are linked together, and cannot be identified the particular source of fluxes.

2.5. Effect Of Phosphorous Fertilization On Plant Material and its CO₂ Assimilation Rate

Climate change has potential to severe extreme climate events such as droughts, and daily temperatures fluctuation, which can easily affect plant physiology and growth rate, impact photosynthetic rate and decrease carbon sequestration. Terrestrial Net Primary Productivity (NPP) is the quantity of remaining atmospheric carbon gained by vegetation through photosynthesis after losses due plant respiration and maintenance (Pan *et al.* 2015). It has been speculated, that plants net primary productivity (NPP) increased with CO₂ rising through improved photosynthesis, but process of increasing biomass also is limited by the nutrients and water availability (Reich *et al.* 2006; Minnen, 2008). Moreover, the enhance of global NPP does not indicate the increase of carbon storage and sink, due to impact of organic decomposition process in soil, which rises with temperature rises, thus lead to the soil CO₂ flux increased (Hou, 2013). For assessments of carbon storage and stock dynamics, it should be considered to include all possible carbon pools, such as aboveground biomass, belowground biomass, and soil carbon. All of these carbon pools play significant role for carbon sequestration in eco-biological system. The FAO study (2017) indicates that Cereals are the world's most important sources of food and since the mid-1960s the

world has managed to raise cereal production by almost a billion tons and new researches predict that by 2030 the developing countries will become increasingly dependent on cereal imports. Wheat (*Triticum aestivum*), common or bread wheat is native to the Mediterranean region and considered as the world's major cereal crop now cultivated worldwide. Import of wheat is expecting to reach 160 million tons in 2030 (FAO, 2015). This volume of production requires investments and has several potential environmental impacts (increasing fertilization, irrigation, monocultures). Investigation of C:N ratio of crop residues is important for modelling carbon (C) and nitrogen (N) dynamics of agricultural systems (Gan *et al.* 2011).

Plant litter decomposition dynamics can vary widely and based on plant species' tissue stoichiometry and requirement for N and P can has large spectrum among plant species within a single ecosystem (Bell *et al.* 2014). Plant productivity and above/below-ground biomass characteristics (such as C:N ratio) are major parameters that impact SOC changes. Investigation of C:N ratio of crop residues is important for modelling carbon (C) and nitrogen (N) dynamics of agricultural systems (Gan *et al.* 2011).

Continual increasing in atmospheric CO₂ concentration has significant effects on photosynthesis and growth of plants, particularly C3 and C4 plants that represent more than 90% of higher plant species (Ibrahim *et al.* 2008). It has been indicated, that rising of atmospheric CO₂ concentrations reduces the oxygenase component of RuBP2 carboxylase/oxygenase, that can lead to increases in photosynthesis (Teramura *et al.* 1990; Sage and Kubien, 2007) and providing availability of carbon skeletons to produce more biomass (Dong *et al.* 2014). Carbon uptake via photosynthesis produces the gross primary productivity (GPP), considering the autotrophic respiration (Ra) of the plants consumed for growth and sustainability, and the remaining carbon is referred to as net primary productivity (NPP) (Hou *et al.* 2013), but it is unclear whether grow of NPP will play a significant part in mitigating atmospheric CO₂ (IPCC, 2007). The maize C4

photosynthetic pathway has a quite slight physiological difference from the wheat C3 pattern, but C4 has certain pros such as ability to decrease their stomatal width and reduce water transpiration and fixing CO₂ at high rates equal or even greater than C3 (Tippie and Pagani, 2007).

Phosphorus (P) is a main nutrient for plants and is highly demanded in many cells and organelles compounds and reaction. The P deficiency similarly negatively impacts growth of C3 and C4 plant species (Jacob and Lawlor, 1991). The photosynthetic carbon reduction (PCR) cycle is common to all plants (C3, C4, and crassulacean acid metabolism [CAM]) (Malkin, 2000) and orthophosphate (Pi) concentration outside the chloroplast could have an effect on the PCR cycle (Rychter and Rao, 2005). Orthophosphate (Pi), CO₂ and H₂O, are primary substrates of photosynthesis (Sivak and Walker, 1986), there carbon is fixed via the PCR cycle in the chloroplast, and then moved to the cytosol as triose phosphate (triose-P) (Rychter and Rao, 2005). The photosynthesis rate is dependent on the ATP/reductant (NADPH, NADH, and ferredoxin) balance (Noctor and Foyer, 2000), and P deficiency imposes limitation on photosynthesis process (Pieters *et al.* 2001) by decreasing the of CO₂ infusion from the atmosphere to the chloroplasts and reducing Rubisco activity (Rychter and Rao, 2005).

Official reporting on the effects of long-term fertilization on photosynthesis parameters of crop plants are very limited. It was demonstrated, that a 20-year different long-term fertilization with wheat-maize double cropping system has effects on leaf photosynthetic characteristics, such as both flag leaf and canopy photosynthetic abilities and yield levels improve with the NPK treatments (Jiang *et al.* 2004). Lovelock *et al.* (2006) demonstrated that P-fertilization did not significantly affect photosynthetic rates and carbon fix and rates of photosynthetic carbon gain was barely correlated with P concentrations in Dwarf *Rhizophora mangle* L. trees (C3 plant). The significant positive effect on the net photosynthesis rate in C3 sweetgum trees (*Liquidambar styraciflua* L.) was found only if Nitrogen and Phosphorus were applied together (Chang, 2003). A field experiment under

semi-arid Mediterranean climatic conditions determined the positive effects of inorganic combined organic/inorganic fertilization on growth, photosynthesis and yield of a sweet maize crop (*Zea mays L.* F1 hybrid Midas), and specified that combined organic and inorganic fertilizers resulted in higher increase in photosynthetic rate and stomatal conductance compared with those under inorganic fertilization (Efthimiadou *et al.* 2010)

In previous work sections, the role of different classes of soil organic carbon as well as the amount and quality of SOC in regulating the chemical and biological characteristics of soil, which are directly linked to carbon sequestration problems and CO₂ emission was reviewed. Fractionation of organic matter in soil into different pools, their specific biological availability and turnover rates may describe in detail the dynamic of soil organic matter content and its quality under specific fertilization treatments. A better understanding of all possible parameters that are influencing soil carbon index will contribute to enhance the positive effects of land practice and crop residues on agricultural soils carbon sequestration, and diminish the negative effects such as mineralization and erosion, thereby promoting sustainability of crop-production. Understanding balance of phosphorus and organic matter plays major role for developing new phosphorus management strategies to successfully dealing with rising atmospheric CO₂ concentrations. It was well documented by several researchers that soil managements; climate and soil properties are the major factors affect soil carbon budget and carbon flux.



3. MATERIALS AND METHODS

3.1. Location and Experimental Layout

The study was conducted at the Cukurova University, Research and Experimental Station of the Soil Science Plant Nutrition Research Center in the Mediterranean region of Adana, Turkey (37-00.47.75 N lat. 35-2131.92 E alt. long. 33 m.a.s.l.). Predominant soil series are Arik. The present southern and eastern Mediterranean soils usually are included to Calcisols group with substantial secondary accumulation of lime. By Soil Taxonomy USDA (2015), the research areas classified as Xerolls with xeric moisture regime. According to the Soil Survey Staff USDA (2015) criteria, the Çukurova region soil moisture regime is xeric (moisture regime, characterized by rainfall occurring immediately after the winter solstice, and followed by a significant dry period after the summer solstice) and the soil temperature regime is thermic (mean annual soil temperature between 15 and 22° C) or occasionally mesic (8–15° C), that is, intermediate between the temperate regions and tropics. The regional climate is typical Mediterranean with long-term average annual air temperature of 19.1°C (ranging from 14.2°C in January-February to 25.5 ° C in July-August) (Table 3.1) and precipitation of 670.8 mm (Anonymous, 2016 Turkish Meteorology data). About 80% of the annual precipitation is received between November and April, with a mean annual humidity of 66% (Anonymous, 2008). Arik soils series are Vertisol with A-C horizon, located on relatively old alluvial soils, the entire profile is clay textured and have high level of calcium carbonate (Çelik *et al.*, 2011; Gülez and Şenol, 2002). Soil series consisted of 50% clay, 32% silt and 18% sand in average depth of 0-30 cm, pH 7.82, total salt content 0.02% (Çelik *et al.*, 2009), lime content 24.4%, SOC content 0.88% and volume weight 1.31 g.cm⁻³ (Table 3.2).

The experiment was established in 1998, and continued through 2016-2017, with two consecutive crops a year: maize in summer and wheat in winter. The experiment was composed of 12 plots laid out in a randomized block design

with three replications and plot dimensions of 10x20 m. The four treatments were as follows: (i) P0 - control, (ii) P- 50 kg P_2O_5 ha⁻¹, (iii) P-100 kg P_2O_5 ha⁻¹, and (iv) P- 200 kg P_2O_5 ha⁻¹ as 3Ca (H₂PO₄)₂·xH₂O. In addition, 160 kg N ha⁻¹ as (NH₄)₂SO₄ for wheat and 200 kg N ha⁻¹ as (NH₄)₂NO₃ for maize were applied along with a uniform application of 50 kg K ha⁻¹ as K₂SO₄. Annually, the inorganic fertilizers were uniformly spread on the soil surface just before sowing and incorporated into the surface of 0- to 15-cm layer with a disc harrow.

Since the experiment was established from 1998 to present time, roots residues of maize and wheat were not removed, and were incorporated by moldboard plowing to 15-cm depth after each harvest. In each growing season, maize was sowed in early June and harvested around 15-30 October, following wheat was sowed around 1-15 November and harvested during 15–31 May. A sprinkler irrigation system (overhead irrigation) was used. Each irrigation lasted for 150 min and was 50 mm (500 m³ ha⁻¹) with an irrigation frequency of 3-7 days, depending on rainfall. The weeds were prevented by rotary hoe during the experimental period. The weeds plant on the wheat/maize row were eliminated manually



Figure 3.1. Location of used area in Cukurova University research field

Table 3.1. Weather Data record during experimental cycle

	2016							2017		
	Aug	Sep	Oc	No	De	Ja	Fe	Mar	Ap	May
Maximum										
Temperature av (C°)	33	30.1	24.1	23.2	17.1	13.9	17	21.1	24.7	27.4
Minimum										
Temperature av (C°)	26	24.2	19.2	11.4	7.2	5.1	5.6	10.3	13	17.1
Precipitation av(mm)	0	2	16	58.4	131	5.8	0.40	7.3	7.3	5.1
Relative Humidity (%)	70.1	68.7	70.3	61.3	66.8	62.1	50.7	62.8	60.7	68.8

Source: Turkish state meteorological service (2016-2017)

Table 3.2. Soil initial Physical, Chemical and Biological characteristics of Arik Soil Series

Properties	Unit	Depth 0-30
Clay	g kg ⁻¹	535±15
Silt	g kg ⁻¹	291±35
Sand	g kg ⁻¹	174±44
Soil organic carbon	%	0.89±0.08
Inorganic carbon	%	24.4±0.43
Total nitrogen	%	0.09±0.01
CEC	Cmol kg ⁻¹	35±1.00
pH (1:2.5)	H ₂ O	7.57±0.55
Salt	%	0.03±0.03
P	mg kg ⁻¹	15.35±1.64
K	mg kg ⁻¹	1132.3±15.3
N°. mycorrhizal spores	10g ⁻¹ soil	89.7±24.03

Mean of two replicates ±S.D.

CEC: cation exchange capacity

3.2. Soil Sampling and Analysis

The soil samples had been collected from field and air-dried ($< 40^{\circ}\text{C}$). A representative part of each soil sample was obtained by cone and quarter method. Soil was sieved through 2-mm mesh was ball-milled and again passed through a 0.25-mm mesh.

Soil P extraction The study has measured plant available P (Olsen, 1954).

Soil inorganic carbon (IC) was measured by pressure-calculator described by Loeppert and Suarez (1996). About 0.5 g of dry soil sample was treated with 10mL of 6M HCl and connected to the gas-tight Scheibler apparatus. Equipment's calibration was performed with CaCO_3 (98% purity).

Soil organic carbon (OC) analyzed by dichromate wet oxidation method with external source of heating according to Tyurin method (Kononova modification (1966)). Soil samples (0.25-mm mesh) were cleaned from noticeable roots, ~ 0.5 gr of soil was taken for analysis and 0.4 N potassium dichromate+ H_2SO_4 (1:1 v/v) was added. Samples were heated under 120°C for 30 minutes and titrated the remnant $\text{Cr}_2\text{O}_7^{2-}$ by 0.2 M ferrous ammonium sulfate (Mohr salt). Soil organic C content (%) was calculated from the difference in Mohr salt used between a blank ($\text{K}_2\text{Cr}_2\text{O}_7$) and the soil sample. All soil samples and blank $\text{K}_2\text{Cr}_2\text{O}_7$ were measured in three replication. The presence of carbonates in the soil does not affect the results of carbon determination by this method.

Soil labile carbon fraction, permanganate oxidizable carbon (POxC, active) was measured by standard protocol with 0.02 N potassium permanganate (Weil *et al.* 2003). The 0.02 M KMnO_4 /0.1 M CaCl_2 solution was produced according to Blair *et al.* (1995) and Weil *et al.* (2003) with sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$) as a standard. The KMnO_4 and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were weighed and dissolved in 2 L water. The pH was adjusted to 7.2 by adding 0.1 M NaOH. Briefly, 2.5 g air-dried soil sample was weighed in a 50-mL centrifuge tube. A volume of 20mL 0.02 M KMnO_4 was added and this suspension was subsequently shaken for 2 min

at 240 rpm. After shaking, the suspensions were allowed to settle for 10 min, then the tubes were opened and 2-mL volumes were transferred by pipetting directly beyond the surface of the solutions into separate flask. Samples was reading by Spectrophotometer UV-160 Shimadzu (550nm).

Carbon management index (CMI): The Carbon Management Index (CMI) was computed by following procedure introduced by Blair *et al.* (1995):

- a) Carbon Pool Index (CPI) : the difference of carbon in soil with treatments (P50, P100, P200), compare to same soil without treatments (P0):

$$\text{Carbon Pool Index (CPI)} = \frac{\text{sample SOC } \text{mg g}^{-1}}{\text{reference SOC } \text{mg g}^{-1}} \quad (3.1)$$

- b) Carbon Lability Index (CLI) is calculated as follows:

$$\text{Lability of C (L)} = \frac{\text{C in fraction oxidized by KMnO}_4 \text{ (mg labile C g soil}^{-1}\text{)}}{\text{C non-labile (mg labile C g soil}^{-1}\text{)}} \quad (3.2)$$

$$\text{Lability Index (LI)} = \frac{\text{CLability of C in sample soil}}{\text{Lability of C in reference soil}} \quad (3.3)$$

- c) The Carbon Management Index (CMI) is then calculated as follows:

$$\text{Carbon Management Index (CMI)} = \text{C Pool Index} \cdot \text{Lability Index} \cdot 100 \quad (3.4)$$

$$\text{CMI} = \text{CPI} \cdot \text{LI} \cdot 100$$

The analysis of Fulvic, Humic acids and Humin were conducted according to Ponomariova and Plotnikova methodic (1980) and includes sequential pouring the same soil samples (about 10 g) with different solvent:

Humic acids.

Fraction 1 - directly soluble in 0.1N. NaOH, free and associated with mobile sesquioxide.

Fraction 2 - soluble in 0.1N. NaOH in decalcified soil and associated mainly with calcium.

Fraction 3 - soluble in 0.02 N. NaOH at 6 hr heated in a water bath and associated with clay minerals and resistant sesquioxide.

Fulvic acids.

Fraction 1a - soluble in 0.1N. H₂SO₄ with decalcification, free and associated with mobile sesquioxide.

Fraction 1 - directly soluble in 0.1N. NaOH and associated with the fraction 1 of humic acids.

Fraction 2 - soluble in 0.1N. NaOH after decalcification and its associated with fraction-2 of humic acids.

Fraction 3 - soluble in 0.02 N. NaOH at 6 hr heated water bath and associated with the fraction-3 of humic acids.

For a more complete extraction (particularly from soils with a high content of exchangeable calcium and CaCO₃) a preliminary treatment of the soil with dilute acid (2% HCl) was conducted.

After each step, from soil extraction was aliquoted (10-50 ml), dried and analyzed by same Kononova modification of Tjurin methodic (1966) for total organic carbon. Second aliquot was taken for acidification (with 1N H₂SO₄) and precipitation of HA, filtered, dried and analyzed by Tjurin methodic for total organic carbon of humic acid.

Soil nitrogen concentration as total Kjendal nitrogen content was analyzed with automatic nitrogen analyzer VELP (Scientifica, Italy) and Kjeldahl digestion and a steam distillation unit (Model UDK132, VELP Scientifica, Italy). Sample digestion was carried out according to the reference procedure (Cunniff, 1996). Briefly, a 5.00 gr soil sample was digested with 7.00 mL of concentrated

H₂SO₄ in a block digester at 420° C for 180 min. The digest was allowed to cool down before taking to analysis by automatic VELP Kjeldahl titrimetric method.

The carbon dioxide flux measurement under field conditions was conducted by automated soil CO₂ flux chambers LI-COR model LI-8100A (8100-103, LI-COR, Lincoln, NE, USA). The CO₂ emissions were measured *in situ* twice per each observation month, using a portable analyzer to determine accumulation of CO₂ in a vented chamber that enclosed an area of 177 cm². The closed-chamber method is a common approach used to estimate the soil flux of CO₂ (F_c, μmol m⁻²s⁻¹). In this chamber based method, a portion of air is flowing from a chamber to an infrared gas analyzer (IRGA) and then transfer back to the chamber. The chambers run with a pneumatic system, which raises the chambers up between repetitions to perform an equilibration with ambient CO₂ concentrations. Flux is determined considering chamber volume, soil surface area, air temperature, atmospheric pressure, and the rate of CO₂ concentration increase inside the closed chamber (μmol mol⁻¹ s⁻¹) which has been automatically closed the soil surface for required period of time (2 minutes). The each one from total 15 chambers was set on a polyvinyl chloride (PVC) soil collar with 20 cm in diameter, which was inserted 6 cm into the soil in each treatment (12) and 3 control chambers were installed between treatment plots in a bare soil row with no cover crop. Fluxes of CO₂ (expressed as F μmol CO₂ m⁻² s⁻¹) were analyzed from the rate of increase in CO₂ in the closed chamber during a 120-s deployment period. Flux is determined from the rate at which gas concentrations change inside the chamber and following formula (5):

$$F_{CO_2} = \frac{Flow}{A} (CO_{2Sample} - CO_{2Reference}) \quad (3.5)$$

Where:

$Flow$ = flow rate passing through the chamber;

A = soil area inside of chamber;

$CO_{2Sample}$ = sample air CO_2 concentration;

$CO_{2Reference}$ = reference air CO_2 concentration.

Flux measurements were conducted through 2 seasons each 2 weeks. In each treatment spot, three measurements were taken to estimate the mean flux from each plot. All measurements were made during day time around 0800 to 1000 *am* (UTC +3). Soil moisture and temperature were measured simultaneously in each plot using a portable device attached to the analyzer. To convert CO_2 flux to $C\ t\ ha^{-1}$ the μmol was used as gram, where 1 mol of CO_2 contain 1 mol as 12g of carbon and second was converted to hours, than to required time period (3 months)

3.3. Plant Material Analysis

The Photosynthesis - Assimilation rate measurement. Carbon dioxide net assimilation (Photosynthesis rate) was measured *in situ* by gas exchange methodic using the LI-6800 Portable Photosynthesis System (LI-COR, Inc. (2016) LI-6800 Portable Photosynthesis System, Software Version 1.0. LI-COR, Inc. Lincoln, NE), which provides characterization leaf-level CO_2 and H_2O flux, stomatal conductance, intercellular CO_2 concentrations, carboxylation and light use efficiencies, and CO_2 and light compensation points. The LI-6800 equipment maintains ambient condition, controls the leaf chamber environment at the set points with low variance ($n = 3$; $\pm$ standard error of the means), an automated leaf-level control of temperature keeps the biochemistry constant for the entire response as transpiration cooling changes with stomatal conductance. Chamber's conditions there are following: fan speed of 10 000 rpm, flow rate of $600\ \mu mol\ mol^{-1}$,

overpressure of 0.2 kPa, vapour pressure deficiency (VPD) at leaf (VPD_{leaf}) of 1.2 kPa (controlled by the LI-6800), saturating irradiance of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Simultaneously, light intensity, ambient humidity and temperature were recorded by the LI-6800. Measurements from the youngest fully expanded or flag leaves were taken once per month for 3-to 5-month-old maturity stage and 2-to-3 for maize plant, hourly from 0800 to 1000 *a.m.* (UTC +3). Net CO₂ assimilation rate were measured by analyzing three leaves of each selected treatment spot during spring (April-May) for wheat plant, during summer and autumn (August - September) for maize plant with a LI-6800 LI-COR equipment. The used equipment is an open gas exchange system, and its measurements of photosynthesis and transpiration rates are based on the differences in CO₂ and H₂O in an air flow entering and exiting the leaf cuvette with a following formulations (6, 7):

$$CO_2 \text{ Assimilation} = \frac{\text{Flow} \times (\text{Sample } CO_2 - \text{Reference } CO_2)}{\text{Leaf Area}} \quad (3.6)$$

$$H_2O \text{ Transpiration} = \frac{\text{Flow} \times (\text{Sample } H_2O - \text{Reference } H_2O)}{\text{Leaf Area}} \quad (3.7)$$

The plant biomass and C, N contents: Plants were harvested at last growth stage (harvest). Biological yield per hectare was assessed as above, belowground and yield dry matter weight. For a complete dry weight determination, samples were oven-dried at 65°C until constant dry weight. The oven-dried and meshed plant samples (above and below ground) were analyzed for total carbon and nitrogen content by dry combustion with Thermo Scientific™ FLASH™ 2000 Organic Elemental Analyzer (OEA) (Thermo Fisher Scientific S.p.A. Milan, Italy). Plants materials were analyzed through an elemental analyzer with automatic autosampler and the C, N content was calculated automatically by the dedicated "Thermo Scientific Eager Xperience Data Handling Software".

Determination of phosphorous plants shoots and roots materials was conducted by using Perkin Elmer inductively coupled plasma optical emission spectrometer (ICP) using dry ashing method (550° C) with 6 N HCl as a digestion solution.

The total area carbon content per plot was calculated by multiplying the carbon values with the biomass values (multiplying carbon content with dry biomass amount), then extrapolated per hectar.

Plant carbon storage calculated as ton C ha^{-1} :

$$C_{\text{plant}} = (\text{Biomass t ha}^{-1}) * C\% / 100$$

Plant CO₂ storage was calculated by

$$\text{CO}_{2\text{plant}} = 3.66 * C_{\text{plant}} \text{ t ha}^{-1}$$

3.4. Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model-univariate procedure from SAS 9.4. (SAS Inc., Cary, NC, USA). Significant relationships between all variables were tested with Pearson's correlations, and LSD's multiple rang multiple range were tested. Significance for all variables were established at $p < 0.05$ for all analyses. Tables, graph bars of data was carried out using Excel Office 2013 product (Microsoft Software Inc USA). Data is presented as means and standard deviation.

4. RESULTS AND DISCUSSION

4.1. Maize Growing Season

4.1.1. Effect of Long Term Phosphorus Application on Plant Biomass

Effect of long-term phosphorus (P) application on maize biomass demonstrated in Table 4.1. The highest shoot dry weight (SDW) biomass (16.1 t ha⁻¹) was obtained with 100 kg P₂O₅ ha⁻¹ treatments and the lowest results was found in control (13.2 t ha⁻¹) treatment. Significant impact of P fertilization on SDW of maize plant was found by statistical analysis. Root dry weight (RDW) biomass was determined to be significantly ($p<0.05$) different among treatments. Control treatment had 1.56 t ha⁻¹ RDW and 200 kg P₂O₅ ha⁻¹ treatments have the highest RDW biomass (1.98t ha⁻¹). Although, with increasing P dosages increased grain yield, however this increase was not statistically significant. Also long-term phosphorus application had no significant impact on maize total biomass yield (Table 4.1). According to *Türkiye İstatistik Kurumu* (2013), local average maize yield is 8.95 t ha⁻¹ (TUIK, 2013), however, present study yield was affected by pathogenic fungus *Ustilago maydis*. The total maize biomass demonstrated weak significance in increasing from control level up to 100 kg P₂O₅ ha⁻¹ and decreasing in 200 kg P₂O₅ ha⁻¹ level. In general, shoot, root and grain yield increased with the increase of P dosages.

Table 4.1. Maize plant biomass response to different phosphorus fertilization levels

P-level	Maize Biomass DW			
	Roots	Shoots	Grain	Total biomass
	t ha ⁻¹			
0	1.56 ±0.1c	13.2 ±0.8b	5.14 ±1.6b	19.9 ±2.6b
50	1.70 ±0.1cb	14.2 ±0.6ba	6.9 ±0.5ab	22.5 ±1.2ab
100	1.83 ±0.1ab	16.1 ±0.4a	7.71 ±0.1a	25.6 ±0.7a
200	1.98 ±0.4a	14.6 ±0.2ba	7.1 ±0.4ab	23.5 ±0.8ab
<i>F</i>	6.91	5.42	NS	3.77
<i>Pr>f</i>	0.013	0.024		0.059

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

4.1.2. Effect of Long Term Phosphorus Application on Maize Biomass C, N, P concentration, their ratios and biomass nitrogen uptake

Carbon (C) is a structural element and represents near in between 40-45 % of the plant's dry mass. The average total C content in maize plants is 42.4 % for shoots (Table 4.2). All treatments are not significantly different among each other. With 50 kg P₂O₅ ha⁻¹ dosage maximum C % concentration was obtained and in control plots minimum C concentration was obtained. Similarly, maize roots data shows averagely 42% C content with highest value at 50 kg P₂O₅ ha⁻¹ treatments, although all treatments were found to be not significantly different.

Maize shoots total nitrogen (TN) content was significantly ($p < 0.05$) affected by increasing P treatments (Table 4.2.). The highest TN for 200 kg P₂O₅ ha⁻¹ treatment was 1.23% and lowest TN was 0.52% for control treatment. On the contrary, maize root's nitrogen concentration was found to have similar results among treatments, with a range of 0.5-0.7%.

Table 4.2. Maize plant tissue characteristics response to different phosphorus fertilization levels

		Maize plant tissue characteristics				
P-level		C%	N%	P%	C:N	C:P
Shoot	0	39.4 ±1.5a	0.52 ±0.1c	0.08 ±0.02c	75.8	486.44
	50	42.3 ±1.1a	0.85 ±0.2b	0.11 ±0.02cb	49.1	384.54
	100	41.3 ±2.1a	1.22 ±0.2a	0.15 ±0.02b	34.2	271.12
	200	41.6 ±1.4a	1.23 ±0.1ab	0.24 ±0.05a	35.3	177.82
	<i>F</i>	<i>NS</i>	<i>8.32</i>	<i>10.01</i>		
<i>Pr>f</i>			<i>0.01</i>	<i>0.004</i>		
Root	0	42.7±1.01a	0.48 ±0.04a	0.11 ±0.03a	88.15	384.54
	50	43.1 ±1.47a	0.64 ±0.05a	0.08 ±0.03a	67.78	529.63
	100	41.3 ±0.68a	0.7 ±0.09a	0.06 ±0ab	63.41	646.33
	200	40.8 ±3.54a	0.7 ±0.12a	0.09 ±0.02a	62.88	477.11
	<i>F</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>		
<i>Pr>f</i>						

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: 50 kg P_2O_5 ha⁻¹; 100: 100 kg P_2O_5 ha⁻¹; 200: 200 kg P_2O_5 ha⁻¹

Expectedly, P content in maize shoot was found to be significantly ($p < 0.05$) affected by P fertilization, with gradual increase in results from control (0.08 P %) to the highest result (0.24 P %) was found at 200 kg P_2O_5 ha⁻¹ level application. However, the below ground plant parts did not significantly differ, and the highest P values were found in control and 200 kg P_2O_5 ha⁻¹ dosage application.

After measuring plant tissue C, N and P concentration C:N and C:P ratios were calculated and data are presented in Table 4.2. The effects of P fertilizer supplementation on C: N and C: P ratios of the plants were decreased with P application increases. In maize plant C: N ratio shows that with increasing P fertilize levels C: N ratios are decreased in both shoot and root measurements. For control treatment, upper part shoot C and N concentration ratio C: N was 75.8 and

decreased to 35.3 with 200 kg P_2O_5 ha⁻¹ fertilizer application (Table 4.2). The C: N ratio of maize roots has the similar pattern with shoot C: N ratios. In control treatment C: N ratio was the highest 88.1, with increasing 50, 100, and 200 kg P_2O_5 ha⁻¹ treatment ratio gradually decreased 67.7, 63.4 and 62.8 respectively. For other crop species, C:N ratio growth with plant maturity and low-quality plant residue having high C:N ratios, which is resulting slow release of nutrients (Seiter and Horwath, 2004). The plant detritus with a high C:N ratio (>40) are likely to have a different effect on nutrient mineralization during degradation processes than decomposed materials with a low C:N ratio (<40). A relative excess of N in the plant can improve dry matter allocation into the shoot due to the ability of balancing between C and N (Natr and Lawlor, 2005). Drinkwater *et al.* (1998) indicated that low C: N residues enhance C retention in soil. Typically, cereal straw may have a C: N ratio around 100, while ratios <25 are more preferable for microbial decomposition in the way to prevent N deficiency (Roy *et al.* 2006). Lange *et al.* (2015) indicated that higher-quality (lower C: N ratio) of litter will be also utilize rationally by the microorganisms thus apparently stabilizing more of this litter as soil organic carbon (SOC).

Stoichiometry of C: P of higher plant communities is broadly varying, and may have broad ratios C: P from 100 to more than 1100. Maize shoot and root C: P ratio decreased drastically with increasing P doses increases. The C: P ratio in control plants was 486.4 and decreased to 177.8 with 200 kg P_2O_5 ha⁻¹ application. In below-ground C: P ratio shows the lowest value in control treatments 384.5 due to relatively same low C and P content and 477.9 in 200 kg P_2O_5 ha⁻¹ application.

Maize grain's tissue characteristics response to different phosphorus fertilization levels represented in Table 4.3. Maize grain demonstrated to have higher levels of C and N, than another plant parts. The highest carbon content was found in treatments 100 kg P_2O_5 ha⁻¹ and 200 kg P_2O_5 ha⁻¹ (45.6% and 48.2% respectively), however, results were not statistically different. Nitrogen

concentration also was found to be not statistically different among treatments and demonstrated 1.39% in control plots and mean 1.59% in all another dosages.

Table 4.3. Maize grain's tissue characteristics response to different phosphorus fertilization levels

P-level	Maize grain's C and N concentration		
	C %	N %	C:N
0	44.7±5.3a	1.39±0.13a	32.13
50	43.9±3.6a	1.59±0.2a	27.63
100	45.6±1.9a	1.56±0.1a	29.34
200	48.2±1.2a	1.57±0.15a	30.87
<i>F</i>	NS	NS	
<i>Pr>f</i>			

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: 50 kg P_2O_5 ha⁻¹; 100: 100 kg P_2O_5 ha⁻¹; 200: 200 kg P_2O_5 ha⁻¹

Maize biomass total nitrogen (TN) uptake demonstrated in Table 4.4. Maize shoot biomass TN uptake was significantly affected by the P application level and it has been shown that the higher values in 100 and 200 kg P_2O_5 ha⁻¹ was obtained (196 kg ha⁻¹ and 179 kg ha⁻¹ N respectively), and in control plots significantly lower values (69.8 kg ha⁻¹ N) was obtained.

Similarly, maize root biomass TN uptake was significantly affected by the fertilization and demonstrated higher values in 100 and 200 kg P_2O_5 ha⁻¹ (12.1kg ha⁻¹ and 13.3 kg ha⁻¹ respectively), and lower values in control plots (7.5 kg ha⁻¹).

Table 4.4. Maize biomass nitrogen uptake (mg kg^{-1}) response to different phosphorus fertilization levels.

P-level	Maize Biomass Nitrogen Uptake			
	Shoot's	Root's	Grain's	Total
	N uptake			Biomass N uptake
	kg ha^{-1}			
0	69.8 $\pm$ 11.1c	7.51 $\pm$ 0.2b	72.1 $\pm$ 23.1b	149.6 $\pm$ 34.5b
50	123.6 $\pm$ 27.7bc	10.1 $\pm$ 0.3a	110.8 $\pm$ 17.5a	245.1 $\pm$ 43.8a
100	196.8 $\pm$ 25.7a	12.1 $\pm$ 1.7a	120.1 $\pm$ 8.1a	329.4 $\pm$ 35.3a
200	179.1 $\pm$ 8.2ab	13.3 $\pm$ 2.2a	109.2 $\pm$ 4.1ab	302.4 $\pm$ 10.4a
<i>F</i>	6.33	5.17	2.99	8.11
<i>Pr>f</i>	0.0166	0.0282	0.091	0.0083

Total N uptake was calculated on base on kg per hectare. Mean of three replicates $\pm$ S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: 50 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$; 100: 100 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$; 200: 200 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$

Nitrogen uptake of maize grain were tended to be increased with P fertilization application increased, and higher values was found in 100 and 200 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ dosages application, and the lowest was found in control plots. Total maize biomass nitrogen uptake showed to be significantly affected by P fertilization, and demonstrated gradually increased among treatments.

4.1.3. Effect of Long Term Phosphorus Application on Plant C-CO₂ Uptake and Net CO₂ Accumulation Rate

Carbon allocation in above- and below-ground parts of plants is one of C cycling processes, however the carbon sequestration in above and below-ground parts currently is still poorly understood, and such information is needed to improve the assumption of the C budget and stocks to provide the basis for land management at a regional scale (Chen *et al.* 2015).

Maize shoot biomass C uptake increased with increasing P fertilizer levels, however this increase was not significant (Table 4.5). In control plots, shoot's C uptake was 5.23 t ha^{-1} , and the highest in $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ application C uptake was 6.64 t ha^{-1} . Maize roots carbon uptake also does not demonstrated statistically significant differences amount the P levels applications. Although, with $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ and $200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ applications have slightly higher results ($\sim 0.8 \text{ t ha}^{-1}$ both) compare to control treatment, but this differences was not made the fertilizer application significant. Grain's C uptake demonstrated similar values with slight increasing with fertilization level increased.

Table 4.5. Maize plant biomass carbon uptake responses to different phosphorus fertilization levels

P-level	Maize Biomass Carbon Uptake				Total CO ₂ fixation
	Shoot's	Root's	Grain's	Total	
	C uptake			Biomass C uptake	
	t ha^{-1}				
0	$5.23 \pm 0.52\text{b}$	$0.66 \pm 0.07\text{b}$	$2.27 \pm 0.64\text{b}$	$8.16 \pm 1.2\text{b}$	$29.9 \pm 4.4\text{b}$
50	$6.1 \pm 0.45\text{ab}$	$0.73 \pm 0.06\text{ab}$	$3.1 \pm 0.61\text{ba}$	$9.86 \pm 1.0\text{ab}$	$36.1 \pm 3.7\text{ab}$
100	$6.64 \pm 0.34\text{a}$	$0.75 \pm 0.01\text{ab}$	$3.52 \pm 0.1\text{a}$	$10.9 \pm 0.3\text{a}$	$39.8 \pm 1.1\text{a}$
200	$6.1 \pm 0.1\text{ab}$	$0.81 \pm 0.06\text{a}$	$3.37 \pm 0.2\text{ab}$	$10.3 \pm 0.34\text{ab}$	$37.5 \pm 1.2\text{ab}$
<i>F</i>	3.25	2.48	2.35	3.21	3.21
<i>Pr>f</i>	0.08	0.1358	0.148	0.083	0.083

Mean of three replicates $\pm$ S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$; 100: $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$; 200: $200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$

Total C uptake also was calculated and in control plots total C uptake was 8.16 t ha^{-1} and in $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ dosage demonstrated the highest total C uptake 10.9 t ha^{-1} . Although with increasing P levels total C uptake increase, however it was not statistically significant. By using total C uptake, total CO₂ fixation was

calculated and it has been found that increasing P level total CO₂ fixation increased. However, this increase is not statistically significant. Due to higher shoots biomass consequently CO₂ uptake is the highest as 39.8 t ha⁻¹ in 100 kg P₂O₅ ha⁻¹ dosage.

Effect of P Fertilizer Level on Net CO₂ accumulation rate: Since with increasing phosphorus fertilizer application increased total C uptake and CO₂ fixation it is very important for atmospheric CO₂ increasing mitigation. The carbon fixation as CO₂ by plants supply energy and building materials for all life on earth, however despite its huge importance, the C-cycle in agricultural areas still remain difficult to measure and estimation of C balances have only lately been better understood due to field gas exchange measurements availabilities (Scholefield *et al.* 2007). In the present study, the infrared gas analyzer (IRGA) allow quick measurements of CO₂ exchange rates and leaf photosynthetic rates (net assimilation rate, NAR) of field-grown crop canopies were studied. Maize plants net CO₂ assimilation rate and photosynthetic characteristics response to different P fertilization levels demonstrated in Table 4.6. Field measurement were performed twice, on active grow stage (30 days old plant) and stem elongation stage (45 days old plant). It was found, that P fertilization did not have significant impact on maize NAR and demonstrated mean value increased and average mean value was 32.2±2.4 µmol m⁻² s⁻¹. Generally, maize as a C4 plant has higher CO₂ assimilation rates typically <60 µmol CO₂ m⁻² s⁻¹, than C3 plant (Zelitch, 2012).

Table 4.6. Maize photosynthetic characteristics response to different phosphorus fertilization levels

Plant growth stages		CO ₂ Assimilation rate $\mu\text{mol m}^{-2}\text{s}^{-1}$	Transpiration rate $\text{H}_2\text{O mol m}^{-2}\text{s}^{-1}$	Intercellular CO ₂ $\mu\text{mol mol}^{-1}$
1 month	p-level			
growth				
stage	0	27.56±6.6	0.0077±0.004	120.95±39.3
(Active	50	30.61±7.1	0.0069±0.002	85.80±6.02
growth)	100	37.37±3.1	0.0085±0.002	92.16±11.8
34° C	200	34.07±5.6	0.0087±0.0007	103.07±44.7
	<i>p</i> < .05	<i>ns</i>	<i>ns</i>	<i>ns</i>
1.5 months				
growth	0	31.18±2.4	0.003584±0.001	338.19±3.6
stage (Stem	50	33.57±3.4	0.005332±0.003	324.34±8.5
extension)	100	33.95±4.4	0.003145±0.001	332.44±9.3
37 ° C	200	29.94±5.9	0.00202±0.001	339.03±10.05
	<i>p</i> < .05	<i>ns</i>	<i>ns</i>	<i>ns</i>
	0	29.37±1.8	0.00565±0.002	229.57±108.6
	50	32.09±1.4	0.00614±0.0008	205.07±119.2
Mean	100	35.66±1.7	0.00582±0.002	212.30±120.1
	200	32.01±2.1	0.00535±0.003	221.05±117.9

Mean of three replicates ±S.D. NS – not significant at *p* < 0.05 by LSD test. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

Net photosynthesis in maize (low photorespiration and a high net CO₂ assimilation rate) may double for every 10° rise from 15 to 35°; whereas at temperatures >35, reduction in net photosynthesis is related to stomatal closing (Zelitch, 2012). The decline in the photosynthesis rate mostly taken place as response to reducing stomatal conductance. However, in present work, even under

ambient temperature reached 37° C, not significant difference was observed, where decreasing of assimilation rate could be associated with stomatal conductivity reducing.

Ghodrat *et al.* (2013) indicated that, generally, maize NAR is reached maximum on growing and flowering stages and slightly reduces in maturity stages, thus we can assume that found in growing stage NAR of maize will not change till maturity. Maize intercellular CO₂ increased with plant growth (Table 4.6.). Although higher CO₂ concentrations can increase photosynthesis, this benefit was reduced in high temperatures as plants close stomata to conserve water (Ruiz-Vera, 2013). Zhang *et al.* (2013) results indicated that warming maize leaves impacted photosynthesis through the changes in stomatal characteristics including stomatal frequency, stomatal size that may increase the photosynthesis rates of maize plants.

4.1.4. Effect of Long Term Phosphorus Application on Soil Chemical Characteristics

Soil organic matter (SOM) is used as a central indicator of soil health (Weil, 2004) and quality control of soil ability to recover itself under agricultural production process. Soil C cycling is related to the availability of main nutrients: nitrogen (N) and phosphorus (P). However, the role of soil P in affecting soil C turnover and sequestration is partly understood, whereas most soil C accumulation models is using C:N ratios, relying on the N application is prone to increase microbial activity. Most soils in the Mediterranean region are inherently low in P concentration and respond to applied P (Ibrikci *et al.* 2005). The P fertilizer application may significantly improve the soil-C in the cereal cropping system due to an evident increase in the plant root density following P application (Ibrikci *et al.* 2009). Similarly in present field experiment Ortas and Lal (2013) showed that increasing P fertilizer application increased SOC concentration as well.

4.1.4.1. Soil Organic Carbon

Active carbon (Permanganate oxidizable carbon), soil organic carbon, phosphorous and nitrogen content after maize cultivation season demonstrated in Table 4.6. Permanganate-oxidizable C (POXC) was measured for estimation of active organic carbon that might provide information of soil C dynamics and mineralization rate. There have limited studies of examination of P impact on particular fraction of carbon.

In present study, the results of permanganate oxidizable carbon were significantly affected by P fertilization (Table 4.7.). Results shows maximum of active carbon in zero P application plots for 0-15 cm soil depth, and for 15-30 cm soil depth no significant impact was observed. Active carbon content in 0-15 cm soil depth is statistically significant difference among treatments and active C in 15-30 cm depth was not significant.

Although, with increasing P fertilizer rate in 0-15 cm active C content decreased, however, in 15-30 cm depth active C content increased with P fertilizer level increase. The data demonstrates positive correlation between active C and available P in soil after the maize growing season ($r^2 = 0.72$, $\alpha = 0.0079$) (Table 4.10.). When plant allocate a higher quantity of photo-assimilated products belowground this results in releasing more labile exudation substrates by roots (Pausch and Kuzyakov, 2018). Taken into account this suggestion, that active carbon was mostly affected by plant exudates, it was assumed that changes into results was rely on plant type and its biomass.

Table 4.7. Soil characteristics response to the different phosphorous fertilization levels under Maize

depth	P-level	Soil characteristics				
		PoXC mg kg ⁻¹	SOC g kg ⁻¹	P mg kg ⁻¹	N g kg ⁻¹	C:N C:P
0-15cm	0	718.8 ±91.4a	4.63±0.2a	1.61 ±0.03b	0.76 ±0.02b	6.1 2870.7
	50	508.9 ±51.2b	5.36±1.06a	5.93 ±1.01b	0.85 ±0.03a	6.3 952.3
	100	561.2 ±13.5b	4.94 ±0.6a	4.41 ±2.7b	0.80 ±0.02ab	6.2 1558
	200	580.1 ±57.8b	4.65 ±0.7a	15.86 ±5.5a	0.83 ±0.03ab	5.6 351
F		4.44	NS	7.87	NS	
Pr>f		0.04		0.009		
15-30cm	0	343.7±100.8a	5.53 ±1.2a	0.87 ±0.53b	0.8 ±0.012a	7.2 7920.7
	50	459.1 ±109.2a	7.74 ±4.05a	2.12 ±0.09b	0.7 ±0.04a	10.8 3732.5
	100	397.5 ±70.1a	8.13 ±3.7a	1.41 ±0.46b	0.7 ±0.08a	10.5 5658
	200	529.9 ±13.3a	7.49 ±2.08a	8.08 ±5.7a	0.8 ±0.1a	9.4 1657
F		NS	NS	NS	NS	
Pr>f						

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. P0: Control; P50: 50 kg P₂O₅ ha⁻¹; P100: 100 kg P₂O₅ ha⁻¹; P200: 200 kg P₂O₅ ha⁻¹. PoXC – Permanganate oxidizable carbon.

However, below maize biomass was just weakly negatively correlated with PoxC on 0-15 cm soil depth, and positively in lower horizon (Table 4.10.)

In 0-15 cm and 15-30 cm soil depths with increasing P fertilizers SOC content is increasing. In 0-15 cm depth, it has been demonstrated that the average mean value was 4.89g kg⁻¹, and in 15-30 cm soil depth the mean SOC was 7.22 g kg⁻¹ (Table 4.7.). In generally in 15-30 cm have high SOC content than 0-15 cm depth and for both depth, effect of P fertilizer on SOC was statistically not significant. The trend of lower amount of SOC after maize cultivation also was observed in previous 2015-2016 years long-term experiment, however overall SOC results was higher (Appendix 1.).

The SOC comparatively low results at maize growing season may be due to increasing microbiotas and enzymes activity with temperature rise, soil warming causing increases the decomposition of organic matter, enhancing microbial growth and enzyme synthesis (Davidson and Janssens, 2006). Meta-analysis results demonstrated that the increase of soil C storage is significantly positively affected when NPK and P fertilizers were applied over longer durations (>20 years) and indicated that consistent variability in soil C storage was 30–33% when N was added simultaneously with P fertilizer (Zhao *et al.* 2017). Whereas, Bird *et al.* (2011) observed a 20% decreasing of SOM in cultivated grassland soil, compared to uncultivated soil. Present experiment was set up in 1998 and since then SOC in P fertilizer plot slowly increased. Our data are in harmony with results of Zhao *et al.* (2017).

It is challenging to examine the effects of only crop rotation on SOC balancing, due to uncertainty impact of root exudates and climatic seasonal changes. In the 30 years long term experiment of crop straw left on chernozems soil surfaces haven't impact on the C content (Campbell *et al.* 1995). Similarly, Reicosky and Saxton (2007) reported that 30-year continuous experiment with maize plants, leaving or removal of plants residue had no significant impact on SOC contents. Moreover, total C and N, and C: N ratio in the soil remained same

after 30 years experimenting in fertilized treatments (Nitrogen + Plant residue). Da Silva Oliveira *et al.* (2017) reported that high proportions of SOC could be overestimated as Active carbon in permanganate oxidation method.

Carbon management index (CMI) is an indicator of soil organic C dynamics and treatments effect on SOC, based on relationship of in the stable and labile soil organic C under treatments related to the reference of non-treated soil. Initially, CMI was introduced by Blair *et al.* (1995) and explained as calculation of effect of agricultural practices on labile and non-labile C pools. Due to the fact, that CMI index is relationship between labile and non-labile SOC fraction, the results shows hidden impacts of fertilization on SOC, compare to non-treatment samples, which in present study is control P-0 plots (Figure 4.1).

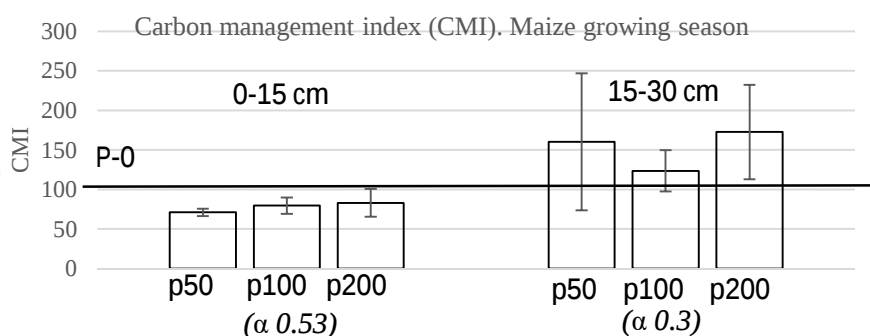


Figure 4.1. Carbon management index response to different phosphorous fertilization level under maize rotation.

Mean of three replicates $\pm$ S.D. α –significance at $p < 0.05$ by LSD. p0: Control; p50: 50 kg P_2O_5 ha⁻¹; p100: 100 kg P_2O_5 ha⁻¹; p200: 200 kg P_2O_5 ha⁻¹

The data below the P-0 level indicate that current treatment has decreased as quality of the SOC (Souza *et al.* 2016) compare to not fertilization treatment. As it shown on figure 4.1. CMI index was higher in 15-30 cm then 0-15 cm depth. The results suggested that for 0-15 horizon, at maize growing season, all phosphorus dosages had similar negative impact on SOC quality, whereas the situation is different for lower horizons, there all treatments demonstrated positive impact on

SOC quality, especially for 200 P_2O_5 kg ha^{-1} dosages, although statistically significant difference was not found. However, in present study it cannot be say if the used treatments have impact of CMI, or another variable, such as weather condition or the fact that seasons 2016-2017 were claimed as the hottest year on record globally by NASA (Potter *et al.* 2017), which can have impact on biological activity and increase SOC decomposition rate.

4.1.4.2. Soil Nitrogen Content

Nitrogen (N) is a major limiting element to agricultural production and the understanding N cycling and C sequestration interaction is crucial in estimation and prognosis of the consequences of particular management practice and further increases in CO_2 emission. The studies of C sequestration is concerned the stabilization processes and soil respiration (SR), microbial decomposition of soil organic matter and "priming effect" responds to N application is relevant to C sequestration. The effects of elevated CO_2 on soil respiration may be influenced by N deposition as well. Despite of fertilization might accelerate initial litter and SOC decomposition, but it may simultaneously promote soil C stabilization (Jackson *et al.* 2017).

In present study, total soil N was not significantly affected by P fertilization for both soil depths (Table 4.7.) within the range of 0.79-0.83 N g kg^{-1} . Increasing P fertilizer level did not made any differences, and statistical results show no correlation between phosphorus fertilization and C:N ratio, meanwhile SOC and C: N ratio have strong positive correlation (Table 4.10.). Soil C:N ratio shows pattern of increasing ratio in maximum phosphorous dosage for 15-30 soil depth in maize rotation (Table 4.7). The 15-30 depth horizons show more theoretically ideal C:N ratio (~9.5) for maize growing season, while upper horizons had a narrow ratio. Due to the fact that carbon (C) and nitrogen (N) are the main component of soil organic matter C:N ratio considered as an indicator of soil fertility, biological activity, carbon storage ability and mineralization rate

(Deng *et al.* 2013). Soil organic matter generally has a C:N ratio in the range of 10–12 for upper soil horizons. However with high C:N ratios, such as a ratio over 30 is considered negative and can lead to N deficiency, where microorganisms will consume it but take N from the soil solution resulting in very low content of N remain and to consume another C and microorganisms start to decomposition of SOM for N obtain (Conklin, 2013). Low C:N ratios indicates the extent ability of soil organisms to decompose organic remains, and to immobilize mineral N and organic carbon is generally more extractable than organic nitrogen, resulting in relative higher concentrations of N (lower C:N ratio) (Piccolo, 1996). The C:N ratios of SOM have often been speculated as parameter of SOM quality; though, explanation of C:N ratio can be challenged due to continuously removing-adding organic material and fertilizer supplementation. In agricultural soil C:N ratios typically are circa 15 to 7, without including labile fraction. The C:N ratios decrease as labile fractions of SOM are lost and SOM aromaticity increases (Wander, 2004). Some data suggested that decrease in soil C:N is not related to the magnitude soil changes in N (Murty *et al.* 2002). Among studies concerning C:N ratios and independently C and N there are reported trend there with increasing soil N mostly C:N ratios had decreased, while management practice C:N had increased ratios with decreasing soil N, which is going along with the CENTURY model where the soil C:N ratio decreasing with increasing soil N availability. Although increasing N content may have acceleration effect on organic matter decomposition, it might appear to retard stable SOM decay in after (Jandl *et al.* 2007).

4.1.4.3. Soil Phosphorous Content

In the present study, the results of Olsen-P in soil are reflecting the dosage of fertilizer application (Tables 4.7.). Increasing P fertilizer doses increased extractable P mg kg⁻¹ soil. In general, both soil depths under high dosage treatments (100 and 200 P kg ha⁻¹), amount of extractable P is showing the

maximum P concentration. In 0-15 cm soil depth, control treatment has 1.1 mg P kg⁻¹ soil and in 200P kg ha⁻¹ application 15.8 mg P kg⁻¹ was found. As expected, phosphorus fertilization has significantly impacted phosphorus content in soil (f value: 7.87; p-value: 0.009), moreover soil phosphorous content demonstrated insignificant positive correlation with maize root's biomass (Table 4.10.). Soil phosphorous in all seasons showed close relationship with the C: P ratio, when C: P is decreasing relative Olsen-P increases. As predicted, P fertilization has significantly impacted P content in soil and thus it's C:P ratio in all rotation for both horizons. Narrowing of C:P ratio with the application P-fertilizers will impact the mineralization rate and, therefore, will increase P-available in soil, where C:P ratio is less than 200:1 mineralization will occur and more than 300:1 immobilization will happen. While immobilization occurs, there is no sufficient amount of P to provide for plants and microorganisms; thus, microorganisms might start to digest another possible P reservoir (Bünemann *et al.* 2004). The P fertilizers application increase plants available P in soil (Djodjic and Mattsson, 2013) and therefore narrowing the C:P ratio. Moreover increasing available P might indirectly affect the C: P ratio by decreasing the plant carbohydrates exudates (Schilling *et al.* 1998). The correlation test demonstrated negative strong correlation between soil P content and C: P ratio for both horizons (Table 4.10.). Wang *et al.* (2015) showed that the pattern of C:P ratio might change in the near-surface bog peat from 800-1000 and increases with depth up to several thousands. It is assumed, that P depletion is occurred at C:P range between 800 and 1200 there the higher the C:P factor in the surface soil the lower amount of initial P remained in the decomposing litter, relative to C (Moore *et al.* 2010). Adding organic C to the soil can increase the ratio of C: P, subsequently increased microbial biomass that is leading to plant-available P decreasing (Marschner, 2007). Cleveland *et al.* (2006) in an incubation experiment, showed that CO₂ flux in soils with carbon + phosphorous amendments was significantly increased than flux from soil with only C amendments, which is

proving the idea that increasing C:P ratios is one a key to suppressing decomposition rates (Vitousek, 1984).

4.1.4.4. Soil CO₂ Flux Under Field Condition

Agricultural soils have significant environmental impact on greenhouse gas (GHG) emissions. Soil CO₂ emission is depending on several factors such as soil organic matter mineralization by soil microbes and root respiration. In general, soil air has a higher level of CO₂ (1–10 %) and lower of oxygen (5–10 %) compare to the atmosphere, due to soil organic matter decay, soil microbial activity and root respiration (Montgomery *et al.* 2000). The rate of CO₂ exchanging from soil to the atmosphere is regulated by the rate of CO₂ production in the soil, the CO₂ concentration gradient and soil characteristics pore size and temperature (Raich and Schlesinger, 1992). Effect of the maize crop growth under different dosage of P-fertilization on soil CO₂ flux were studied under a field condition, results presented in Table 4.8.

The soil–atmosphere CO₂ exchange were measured with the static chamber method, which represents summary of organic C decay, root and microbial respiration, as well as in the litter decomposition and calcium carbonate (CaCO₃) dissolution. All treatments including phosphorous-zero application were compared also with bare soil (without crop-cover) (Appendix 2.), laid near the experimental plots area. In the maize growing season, measurements of soil fluxes were conducted in august-september period, with the highest day atmospheric temperature (mean 34.8°C). The average bare soil flux averagely reached 3.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4.8.). However, flux of bare soil shows a maximum as 6.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the hottest day of measurements (37.6 °C), which is even higher value than fluxes of the treatments plots with cover crop and fertilization. All treatments showed no significant difference among the fluxes and measurement days and kept mean as 6.1 for control, 6.8 for 50 kg P₂O₅ ha⁻¹, 6.2 for 100 kg P₂O₅ ha⁻¹ and 5.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 200 kg P₂O₅ ha⁻¹. However, the fluxes at most hottest day was

higher and reached $8,9 \mu\text{mol m}^{-2} \text{s}^{-1}$. With decreasing ambient temperature and plant reach its maturity, fluxes of all treatments began to decrease. It should be noted, that 50 and 100 $\text{P}_2\text{O}_5 \text{ kg ha}^{-1}$ dosages demonstrated slightly higher fluxes, whereas control and 200 $\text{P}_2\text{O}_5 \text{ kg ha}^{-1}$ show lower results.

Table 4.8. Soil carbon dioxide flux response to different phosphorous fertilization levels under Maize growing season.

CO ₂ flux $\mu\text{mol m}^{-2} \text{s}^{-1}$ under Maize growing season							
Date	1 Aug	15aug	25aug	11sept	25sept	10oct	Mean CO ₂ flux
P-level							
0	7.4±1.1	6.7 ±1.1	8.2±0.9	7.7±1.9	6.7±0.7	5.2±0.6	6.6±0.5
50	8.8±0.2	8.7 ±0.5	10.0±0.6	7.8±2.1	6.4±0.9	4.7±0.3	7.3±0.8
100	9.1±0.9	7.3±0.9	8.9±0.8	6.1±1.6	6±0.3	4.4±0.4	6.7±1.1
200	7.9±1.2	6.76±0.3	8.6±0.9	6.4±0.9	5±0.5	4.2±0.6	6.1±0.8
Bare soil	4.7±0.4	3.82±1.3	3.2±0.05	6.5±0.2	2.6±0.2	3.1±0.1	3.7±0.4
$p < 0.5$	NS	NS	NS	NS	NS	NS	
T° C	36.4°	37.5°	34.3°	37.6°	27.2°	25.1°	

Mean of three replicates per each plot ±S.D. NS – not significant at $p < 0.05$ by LSD test. 0: Control; 50: 50 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$; 100: 100 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$; 200: 200 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$. In the present table "bare soil" is the flux obtained from bare soil laying between the experimental plots without cover crop. The f-ratio and the p-value is calculated only between the main treatments, excluding "bare soil" flux results.

The Pearson correlation test demonstrated that soil SOC content and soil C:N ratio in upper horizon, both have significant positive correlation with soil CO₂ flux (Table 4.10.).

The over use of phosphorus fertilizer application (200 kg ha^{-1}) did not show to have any effect on soil CO₂ flux. Similarly, Motschenbacher *et al.* (2015)

reported that differences of CO₂ flux among tillage and/or crop rotations were determined to be related mostly to crop maturity and soil respiration reducing with plant age, due to decreasing fine root biomass (Oertel *et al.* 2016). Dhadli *et al.* (2015) showed that seasonal rising and falling in CO₂ fluxes were controlled by soil moisture and temperature both, and under optimum condition, emissions were mostly related to amount of nitrogen fertilization. Ahirwal and Maitit (2018) indicated that change in soil CO₂ flux depends on plant and soil characteristics and environmental variables, where soil temperature positively influenced the soil CO₂ flux, while soil moisture, relative humidity and surface CO₂ concentration negatively influenced the soil CO₂ flux.

To investigate the balances between soil-atmosphere gases exchanges and biomass CO₂ obtain were compared, balances of CO₂ gain, and losses per maize growing season demonstrated in Table 4.9.

Table 4.9. Total CO₂ uptake and efflux of experimental area under maize 3 months cultivation period.

Maize growing season CO ₂ gas exchanges		
p-level	Total soil CO ₂ efflux	Total biomass CO ₂ uptake
	t ha ⁻¹ growing period ⁻¹	
0	24.3±2.68	29.9 ±4.4
50	27.0±5.09	36.1 ±3.7
100	24.3±5.1	39.8 ±1.1
200	22.5±4.45	37.5 ±1.2
<i>Bare soil</i>	13.9±3.76	0.0

Calculated from mean data. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

As shown (Table 4.9.), all treatments has positive CO₂ balances per growing season (3 months), where total biomass CO₂ uptake is higher than total soil CO₂ losses from experimental area per cultivation period. The highest

fertilization dosages (100, 200), demonstrated less CO₂ losses due to lesser soil CO₂ flux and higher maize biomass. Absence of P fertilization (control plot) demonstrated lower results then recommended fertilization dosage, but therefore positive CO₂ balances (*Total biomass CO₂ uptake* subtract *Total CO₂ soil efflux*). Bare soil area demonstrated negative CO₂ balance.

It can be concluded, that P fertilization demonstrated to have positive impact on CO₂ balances under the hottest summer maize cultivation period in calcareous soil. Balanced fertilization might have impact on plant biomass and consequently improve CO₂ soil-atmosphere gas-exchange balance.

Correlation test: Additionally, correlation test was performed. The results demonstrated in Table 4.10.

Table 4.10. Correlation test for both soil horizon, Maze season

0-15 cm										
Soil P	Soil P	SOC	PoxC	Soil N	Soil C:P	Soil C:N	Shoot	Root	Grain	CO ₂ flux
1	1									
SOC	--	1								
PoxC	--	--	1							
Soil N%	0.448	--	--	1						
Soil C:P	-0.795**	--	0.583*	-0.633*	1					
Soil C:N	--	0.928**	--	--	--	1				
Shoot	--	--	--	--	--	--	1			
Root	0.547	--	-0.407	--	-0.555	--	0.688*	1		
Grain	--	--	--	--	--	--	0.894**	0.760*	1	
NAR	--	0.761*	--	--	--	0.811**	--	--	--	1
CO ₂ Flux	--	0.613*	--	--	--	0.656*	--	--	--	1
15-30 cm										
Soil P	1									
SOC	--	1								
PoxC	--	--	1							
Soil N%	0.667*	--	--	1						
Soil C:P	-0.598*	--	--	--	1					
Soil C:N	--	0.980**	--	--	--	1				
Shoot	--	--	--	--	--	--	1			
Root	0.548	--	0.620*	--	-0.679	--	0.574	1		
Grain	--	--	0.687*	--	--	--	--	0.553	1	
NAR	--	--	--	--	--	--	--	--	--	1
CO ₂ Flux	--	--	--	--	--	--	--	--	--	1

PoXC – permanganate oxidizable carbon. NAR- plant's net photosynthetic effectiveness in assimilating CO₂.

**: p<0.01; *: p<0.05

4.2. Wheat Growing Season

4.2.1. Effect of Long Term Phosphorus Application on Wheat Plant Biomass.

Long-term phosphorus (P) application significantly ($p < 0.01$) increased wheat plant shoot biomass under field conditions (Table 4.11). In control plot, 11.5 t ha⁻¹ shoot dry weight (SDW) was obtained and in 50, 100 and 200 kg P₂O₅ ha⁻¹ dosage application were produced 14.3, 15.4 and 19.3 t ha⁻¹ SDW respectively.

Table 4.11. Wheat plant biomass response to different phosphorus fertilization levels

P-level	Wheat Biomass DW			
	Shoots	Roots	Grain	Total biomass
	t ha ⁻¹			
0	11.5 ±0.5b	1.08 ±0.16b	3.07±0.4b	15.6 ±1.1b
50	13.3 ±2.2b	1.39 ±0.6a	3.18±0.5b	17.3 ±5.b
100	15.4 ±1.1ab	1.8 ±0.1a	4.70±0.7a	21.8 ±0.8ab
200	19.3 ±3.1a	2.11 ±0.3a	4.91±0.7a	26.3 ±3.3a
<i>F</i>	6.81	3.94	7.41	11.10
<i>Pr>f</i>	0.01	0.05	0.01	0.003

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. P0: Control; P50: 50 kg P₂O₅ ha⁻¹; P100: 100 kg P₂O₅ ha⁻¹; P200: 200 kg P₂O₅ ha⁻¹

The wheat root dry weight (RDW) showed to be significantly impacted by fertilization. The root weight increased with increasing P fertilizer application from control plots 1.08 t ha⁻¹ up to maximum in 200 kg P₂O₅ ha⁻¹ dosages as 2.11 t ha⁻¹. Theoretically, when nutrient availability sufficient, plants allocate some of elements comparatively less to roots, due to optimization hypothesis where sufficient nutrients availability represent less effort is required to acquire this resource (Ågren and Franklin, 2003).

Wheat grain biomass results was also found to be significantly different among the fertilizer dosages treatments, the highest yield (4.91 t ha⁻¹) was observed

in phosphorous 200 kg P_2O_5 ha⁻¹, the lowest grain yield in control plots 3.07 t ha⁻¹. Increasing P dosages statistically significantly increased grain yield ($p < 0.01$).

Total biomass was counted and it has been found that increasing phosphorus level statistically significantly increased total biomass ($p < 0.003$). Control, 50, 100 and 200 kg P_2O_5 ha⁻¹ dosage application produced 15.6, 17.3, 21.8 and 26.3 t ha⁻¹ total biomass respectively.

4.2.2. Effect of Long Term Phosphorus Application on Wheat Biomass C, N, P Concentration, Their Ratios And Nitrogen Uptake

Carbon concentration in wheat plant appears to be statistically not affected by fertilization and shows average value as 41.5% for shoot biomass, and comparatively low C content in roots with mean value of 25.7%. (Table 4.12).

Wheat nitrogen content in shoot parts was ranging from 1.22 to 1.97%, and the highest results 1.97% N was obtained within 200 kg P_2O_5 ha⁻¹ dosage application. However, no statistically significant differences were found in between P dosages treatments. Whereas, the wheat root nitrogen content appears to be significantly affected by P fertilization treatments with 0.43 N% in control and 0.91 N% in highest treatments dosage P 200 kg ha⁻¹ application.

The P concentration was demonstrated to be increased with P increasing dosages. With increase P fertilizer significantly increased shoots P % concentration. Although increasing P dosages increased root's P % concentration, however, increases were not statistically significant. Similarly to maize P allocation pattern, wheat plant has no variety between results in root biomass and close to be significantly different in shoot biomass results, with expected maximum in 200 kg P_2O_5 ha⁻¹ application.

Shoot and root C:N ratio was calculated and it has been found that with increasing P fertilizer C:N ratio decreased. C: N ratio was no changed in between control, 50 kg P_2O_5 ha⁻¹, 100 kg P_2O_5 ha⁻¹ dose application and C:N ratio was ranged between 36-37 respectively. The lowest C: N ratio 21.8 was observed at

200 kg P₂O₅ ha⁻¹ doses application. Similarly, the C:N of wheat root plants changed between control, 50 kg P₂O₅ ha⁻¹ (67.14 and 60.7 respectively) and 100 kg P₂O₅ ha⁻¹, 200 kg P₂O₅ ha⁻¹ (39.4 and 27.8 respectively).

Table 4.12. Wheat plant tissue characteristics response to different phosphorus fertilization levels on carbon, nitrogen and phosphorus concentration

		Wheat plant tissue characteristics				
	P-level	C %	N %	P %	C:N	C:P
Shoot						
	0	42.2 ±0.2a	1.30 ±0.3a	0.10 ±0.04b	36.1	323.5
	50	42.8 ±2.5a	1.30 ±0.4a	0.16 ±0.03b	37.6	237.6
	100	40.5 ±2.2a	1.22 ±0.3a	0.20 ±0.03ab	36.1	215.7
	200	42.3 ±1.8a	1.97 ±0.2a	0.26 ±0.05a	21.8	181.3
<i>F</i>		NS	NS	8.71		
<i>Pr>f</i>				0.0068		
Root						
	0	30.3 ±1.5a	0.43 ±0.05c	0.06±0.02a	67.1	525.6
	50	25.4 ±6.3a	0.41 ±0.03c	0.08±0.02a	60.7	292.6
	100	24.9 ±1.5a	0.64 ±0.04b	0.09 ±0.005a	39.4	270.3
	200	24.6 ±2.3a	0.91 ±0.08a	0.12 ±0.02a	27.8	224.1
<i>F</i>		NS	34.44	NS		
<i>Pr>f</i>			0.0001			

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. P0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

Also C: P ratio was calculated for both above and below ground C and P concentration. In general, with increasing P fertilizer C: P ratio is decreased. Concerning the fact, that wheat shoot's P content was weakly affected by treatments, C:P ratio shows decreasing from control to 200 kg P₂O₅ ha⁻¹ treatment. Stoichiometry of C:P of higher plant communities is broadly varying and may have broad ratios C:P from 100 to more than 1100. In general, root (below ground) C: P ratio is higher than shoot C:P ratio. Wheat below ground biomass C: P ratio for

control was 525.6 and for 50, 100 and 200 kg P₂O₅ ha⁻¹ application ratio was 292.6, 270.3 and 224.1 respectively.

Wheat's grain C and N concentration is represented in Table 4.13. Grain's carbon amount was found to be not statistically different among treatments, with a range of 46.1-48.4 C%. Grain N concentration was significantly affected by fertilization ($p < 0.03$), with the highest value (2.39 N%) in 200 kg P₂O₅ ha⁻¹, and the lowest in control plot was obtained such as 1.64 N% . Consecutively, C: N ratio of wheat grain was found to be the lowest in 200 kg P₂O₅ ha⁻¹ dosage.

Table 4.13. Wheat grain's tissue characteristics response to different phosphorus fertilization levels

P-level	Wheat grain C and N concentration		
	C %	N %	C:N
0	46.1±1.9a	1.64±0.08b	28.09
50	48.2±0.7a	1.66±0.03b	28.89
100	47.2±0.3a	1.88±0.01ab	25.05
200	48.4±2a	2.39±0.26a	20.21
<i>F</i>	<i>NS</i>	5.26	
<i>Pr>f</i>		0.026	

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

Wheat biomass total nitrogen (TN) uptake demonstrated in Table 4.14. Total N uptake was calculated on as kg per hectare. Wheat shoot biomass N uptake was significantly affected ($p < 0.008$) by the P fertilization dosage and shows higher results in 100 and 200 kg P₂O₅ ha⁻¹ (186.7 kg ha⁻¹ and 387.7 kg ha⁻¹ N uptake respectively), with lower values in control plots (126.5 kg ha⁻¹ N). Similarly, wheat root biomass N uptake was significantly affected by the P level

application and demonstrated higher values in 200 kg P₂O₅ ha⁻¹ (18.4 kg ha⁻¹ N), and lower values in control plots (4.05 kg ha⁻¹ N).

The N uptake of wheat grain was found to steadily increasing with fertilization level increased. With higher values in 200 kg P₂O₅ ha⁻¹ dosages (117.2 kg ha⁻¹ N) and the lowest in control plots (50.1 kg ha⁻¹ N), results were significantly dissimilar among treatments.

Consequently, wheat total biomass N uptake was significantly affected by fertilization level and gradually increased with fertilizer dosage increased. The amount of N uptake was tightly correspond with plant biomass.

Table 4.14. Wheat biomass nitrogen uptake response to different phosphorus fertilization levels

P-level	Wheat Biomass Nitrogen Uptake			
	Shoot's	Root's	Grain's	Total
	N uptake			Biomass N uptake
	kg ha ⁻¹			
0	126.5 ±17.1c	4.05±0.2c	50.1±9.1c	180.7 ±23b
50	168.5 ±27.1b	5.54 ±0.2c	51.1±10.2c	222.4 ±61.1b
100	186.7 ±29.9b	11.2±0.09b	88.7±7.01b	281.1 ±49.1b
200	381.7 ±35.7a	18.4±0.14a	117.2±12.5a	518.5 ±68.8a
<i>F</i>	8.11	17.05	13.12	10.35
<i>Pr>f</i>	0.008	0.0002	0.006	0.004

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

4.2.3. Effect of Long Term Phosphorus Application On Wheat Plant C-CO₂ Uptake And Net CO₂ Accumulation Rate

Plant C uptake of above and below ground parts is one of key factor to C sequestration estimation, which assists to improve the measurement of the C

budget. Wheat's biomass and grain and total C uptake, and total CO₂ fixation are presented in Table 4.15.

Wheat shoot's biomass C uptake statistically significant gradually increased with increasing P fertilizer dosages application (Table 4.15). In control plots shoot C uptake demonstrated similar to maize results (4.86 t ha⁻¹), although 200 kg P₂O₅ ha⁻¹ application demonstrated higher C uptake is 8.16 t ha⁻¹.

Wheat roots carbon uptake also demonstrated statistically significance among the P levels applications increasing results from control up to 200 kg P₂O₅ ha⁻¹. Similarly, to above ground biomass, wheat root biomass also demonstrated increasing pattern from control plots (0.32 t ha⁻¹) up to 200 kg P₂O₅ ha⁻¹ (0.56 t ha⁻¹).

Table 4.15. Wheat plant biomass carbon uptake and CO₂ fixation responses to different phosphorus fertilization levels.

Wheat Biomass Carbon Uptake					
level	Shoot's	Root's	Grain's	Total Biomass C uptake	Total CO ₂ fixation
	C uptake				
	t ha ⁻¹				
0	4.86 ±0.24b	0.32 ±0.07b	1.42±0.2b	6.61 ±0.5c	24.2 ±1.9c
50	5.69 ±0.8b	0.33 ±0.1b	1.46±0.2b	7.47 ±0.6bc	27.3 ±3.1bc
100	6.22 ±0.26b	0.43±0.02ab	2.21±0.3a	8.88 ±0.1b	32.5 ±0.5b
200	8.16 ±1.4a	0.56 ±0.13a	2.38±0.4a	11.1 ±1.5a	40.6 ±5.1a
<i>F</i>	7.94	4.31	7.15	13.46	13.46
<i>Pr>f</i>	0.008	0.043	0.019	0.017	0.017

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

The wheat grain's C uptake was found to be significantly affected by fertilization ($p < 0.02$). Total C uptake was calculated and with increasing P fertilizer total C uptake increased, results are statistically significant different among treatments. In control, 50 100 and 200 kg P_2O_5 ha⁻¹ treated plots total C uptake was 6.61, 7.47, 8.88 and 11.1 t C ha⁻¹ respectively. Consequently, total CO₂ fixation was calculated on the base of total plant C uptake and it has been demonstrated that gradual CO₂ fixation increased with fertilizer dosages increasing. The total CO₂ fixations (uptake) were calculated as 24.2, 27.3, 32.5 and 40.6 t CO₂ ha⁻¹ respectively to treatment increased. This results indicate that growth and yields of winter wheat may promote with P application under Mediterranean conditions and, therefore, has positive impact on plant CO₂ fixation.

Effect of P Fertilizer Level on Net CO₂ accumulation rate: Elevated CO₂ increases the rate of photosynthesis in many C₃ species, mostly by rising the intercellular CO₂ concentration, enhancing the efficiency of carboxylation, and reducing photorespiration (Dong *et al.* 2014), as was defined by Michaelis-Menten reactions linked with the carboxylation reactions between CO₂ and ribulose biphosphate (Baldocchi *et al.* 2016). Increased CO₂ concentrations are anticipated to enhance growth of C₃ plants mainly due to CO₂ concentration and Rubisco enzyme relation; such as CO₂ will inhibit the oxygenation reaction thus decrease CO₂ loss and energy costs of photorespiration (Jakobsen *et al.* 2016). Wheat grown might be affected due to decline in photosynthesis rather than a leaf area reduction, also net assimilation rate concluded to be a factor in defining plant growth rate (El-Hendawy *et al.* 2005).

Winter wheat as a C₃ plant is characterized by comparatively low rates of CO₂ assimilation (generally $< 20 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), compare to C₄ maize plant. Wheat plants have high rates of photorespiration and do not have an obvious temperature optimum for net photosynthesis at high light intensity level in air 10-35°C.

In the present study, wheat plant Net CO₂ assimilation rate (NAR) displayed different pattern with different plant maturity stage (Table 4.16). At the mature stage growth (4 month old wheat plant), the NAR showed bigger value 13.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in control plots and in 200 kg ha⁻¹ phosphorous applied plots NAR was 6.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Similar results was demonstrated by Özcan (2014), where in a same geographical location, NAR of wheat plant in a winter season was averagely 9.98 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Despite this, the statistical analysis shows no significant difference between treatments for 4 months old wheat plants NAR measurements. On the 5th months of growth stage, the NAR shows reverse pattern with the highest assimilation rate in P 200 kg ha⁻¹ fertilization rate plots the NAR value was 17.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$, meanwhile the lowest results was found in 50 kg P₂O₅ ha⁻¹ fertilizer applied plots as 11.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Table 4.16. Wheat photosynthetic characteristics response to different phosphorus fertilization levels

Wheat photosynthetic characteristics				
Plant growth stages	p-level	CO ₂ Assimilation rate $\mu\text{mol m}^{-2}\text{s}^{-1}$	Transpiration rate H ₂ O $\text{mol m}^{-2}\text{s}^{-1}$	Intercellular CO ₂ $\mu\text{mol mol}^{-1}$
4 months growth stage (Flowering half-way) 24.6°C <i>p</i> < .05	0	13.11±2.06	0.00548±0.0002	213.47±36.2
	50	9.02±2.7	0.00373±0.003	259.25±78.2
	100	7.43±4.6	0.00235±0.002	247.49±19.6
	200	6.84±2.91	0.00289±0.0008	220.41±24.8
		<i>ns</i>	<i>ns</i>	<i>ns</i>
5 months growth stage (Flowering complete) 25.1°C <i>p</i> < .05	0	14.46±3.08	0.00804±0.004	264.4±48.3
	50	11.66±1.7	0.00586±0.0009	267.71±17.1
	100	16.03±3.3	0.00735±0.0004	256.34±31.8
	200	17.83±2.5	0.00729±0.0007	246.11±17.7
		<i>ns</i>	<i>ns</i>	<i>ns</i>
Mean	0	13.79±0.67	0.0068±0.001	238.93±25.4
	50	10.34±1.3	0.0048±0.001	263.48±4.2
	100	11.73±4.2	0.0049±0.002	251.92±4.4
	200	12.34±5.4	0.0051±0.002	233.26±12.8

Mean of three replicates ±S.D. NS – not significant at *p* < 0.05 by LSD test.
 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

Although rising temperatures may have effect on the increasing rate of photosynthesis, it is relatively has no impact on of C fixation due to deficiency of carbohydrates in the leaves and culms due to increased respiration loss (Morita *et al.* 2005). In wheat plants water transpiration rate (stomatal conductance) has no significant variation between treatments, although has different mean value between plant growth stage, and both indicators have increased, displayed that P fertilization did not impact water use efficiency of plants. In addition, environmental fluctuation in light intensity, humidity and soil moisture and temperature also have influence on stomatal conductance (Herrick *et al.* 2004).

To understand the factors that are limiting net carbon assimilation rates analysis of intercellular CO₂ also is widely investigated. It was hypothesized that gaseous diffusion of CO₂ through the intercellular air spaces (between the substomatal chamber and the chlorenchyma cell walls) markedly decrease CO₂ availability to the mesophyll cells (Parkhurst *et al.* 1988). Farquhar and Sharkey (1994) indicated that stomatal closure considered as reason of photosynthetic rate decreasing just if the intercellular CO₂ partial pressure (C_i) also declined. In the present study, the intercellular CO₂ (expressed in $\mu\text{mol m}^{-2} \text{ s}^{-1}$) did not appear to have significant differences between all treatments for both maturities stages (Table 4.16). However, intercellular CO₂ has grown over time from 235.16 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for 4 months old to 258.63 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for 5 months old plants.

Similar to maize, wheat NAR does not show differences among the fertilizer treatments. Currently, there is no enough data shows how plants net assimilation response fertilization to soils where the P is limiting the photosynthesis. The soil nutrient limitation could prohibit eCO₂-induced biomass increasing and related C storage processes (Arora *et al.* 2013). Ellsworth *et al.* (2017) reported that aboveground productivity components did not significantly increase in response to elevated CO₂ in three years, in spite of 19% increase in leaf photosynthesis under P-limited condition. Moreover, it was suggested that current climate-carbon models should include both N and P limitations to vegetation productivity in estimating future C storage (Ellsworth *et al.* 2017).

4.2.4. Effect Of Long Term Phosphorus Application On Soil Chemical Characteristics Under Wheat Cultivation

4.2.4.1. Soil Organic Carbon

Active fraction (permanganate oxidizable fraction) of soil organic C demonstrated significant differences between treatments in upper horizon, with minimum values of PoXC in control plots (561.3 mg kg⁻¹) and maximum in 200 kg P₂O₅ ha⁻¹ dosage (713.7 mg kg⁻¹) (Table 4.17). At 15-30 cm soil depth, the results

shown the similar values among P dosages applications, which are appear to be higher than in the same soil depth under maize plant growing season.

Taken into account suggestion that PoxC is mostly affected by plant exudates, we assume that changes into results were relying on plant type and its biomass. Statistically positive correlation was found between PoxC and wheat shoots biomass ($r^2=0.778$, $\alpha=0.029$) and a strong correlation between PoxC and wheat roots biomass ($r^2=0.762$, $\alpha=0.03$) (Table 4.21).

The SOC content after wheat cultivation season demonstrated significant positive impact of fertilization for upper horizon (Table 4.17). The control and 50 kg P₂O₅ ha⁻¹ dosages was found to be 5.63 and 5.34 SOC g kg⁻¹ respectively, and 100 and 200 kg P₂O₅ ha⁻¹ shows significantly higher results (6.62 and 6.68 g kg⁻¹ respectively). However, in 15-30 cm depth, the results were found not affected by increasing P fertilization.

The SOC amount was significantly higher after wheat cultivation (Table 4.17), than the maize growing season, which may have several explanation ways. Firstly, the wheat production was conducted on relatively colder seasons, where temperature variety is not increase +25°C, with several days with temperature near +0°C. On the other hand, maize plants were growing under very hot and dry condition with day temperature exceed + 35 °C and night +25 °C. Since wheat is growing in a relatively cold seasons and root exudate may not been degraded quickly and consequently more C might be preserved. With general theory of humification, it is known that the natural areas from which mollic horizons developed are the prairies and steppes that experience relatively dry summer and freezing conditions in regions of the northern hemisphere (Liu *et al.*, 2012; Semenov *et al.* 2013). Also it may be the effect of crop management on second crop. Previously Bakhshandeh *et al.* (2017) indicated that in contrast to fertilization, cultivation of certain crops in the previous season, can promote grain yield increase. Possibly increasing in yield may affect SOC content (Ortas and Lal, 2012).

Table 4.17. Soil characteristics response to the different phosphorous fertilization levels under wheat rotation

depth	P level	Soil characteristics					
		PoXC mg kg ⁻¹	SOC g kg ⁻¹	P mg kg ⁻¹	N g kg ⁻¹	C:N	C:P
0-15cm	0	561.32 ±27.2b	5.63 ±0.32b	2.05 ±1.2b	0.80 ±0.08a	7.1	3582.7
	50	609.01 ±54.9b	5.34 ±0.41b	1.64 ±0.41b	0.80 ±0.03a	6.6	3442.5
	100	627.05 ±47.3ab	6.62 ±0.14a	6.66 ±0.4b	0.82 ±0.04a	8.1	999.3
	200	713.71 ±38.4a	6.68 ±0.64a	20.23 ±4.7a	0.88 ±0.02a	7.6	351.1
	F	4.34	5.25	24.69	NS		
	Pr>f	0.043	0.027	0.0002			
15-30 cm	0	666.48 ±19.6a	6.12 ±0.4a	2.23 ±0.6b	0.74 ±0.01b	8.1	2947.7
	50	546.13 ±94.5a	5.84 ±0.3a	1.32 ±0.07b	0.72 ±0.04b	7.5	4424.5
	100	609.42 ±62.3a	5.9 ±0.5a	8.43 ±2.01b	0.81 ±0.03b	7.2	764.5
	200	653.22 ±45.8a	5.96 ±0.5a	17.11 ±6a	0.89 ±0.02a	6.7	394.1
	F	NS	NS	10.46	7.89		
	Pr>f			0.003	0.009		

mean or unree replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. Means in the same row followed by the same letter represent significant differences ($p < 0.05$) among treatments. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹. PoXC – Permanganate oxidizable

Carbon management index (CMI) demonstrates dynamics of active and passive soil organic carbon fraction (Blair *et al.* 1995) and indicates treatment impact on SOC compare to control. The CMI approach is based on the reliance that the sequence of carbon supply hold on both the size of the passive carbon pool and the contribution of the active fraction, which had to be taken into estimation in calculating the CMI (Moura *et al.* 2017). According to Blair *et al.* (1995), CMI values above 100 demonstrate positive impacts on carbon management and the results lower than the Control result specify that ongoing fertilization has negative impact on SOC quality, compare to untreated plots (Souza *et al.* 2016).

In the wheat growing season, in 0-15 cm depth, compare to control treatment fertilization, 50, 100 and 200 kg P_2O_5 ha⁻¹ application has been demonstrated to have a positive impact on SOC quality. For 15-30 cm depth although with increasing P fertilizer level CMI indict increased however no or slightly negative impact have been noticed (Figure 4.2). All the results had shown no significant differential among fertilizer treatments for both soil depths. This indicated of uncertainties in understanding of interaction among stable and labile C fractions in soil and supported idea of necessities of active fraction observation in a movement of C management.

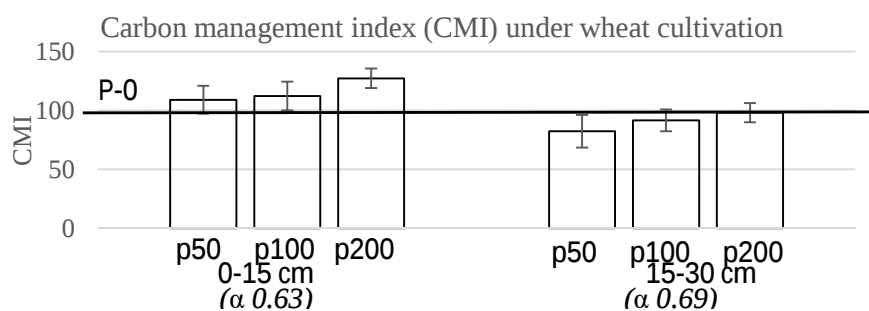


Figure 4.2. Carbon management index response to different phosphorous fertilization level under wheat rotation.

Mean of three replicates $\pm$ S.D. α –significance at $p < 0.05$ by LSD. 0: Control; 50: 50 kg P_2O_5 ha⁻¹; 100: 100 kg P_2O_5 ha⁻¹; 200: 200 kg P_2O_5 ha⁻¹

To further investigation of fertilization impact on soil organic carbon fraction, the humic substances fractionation analysis was performed. However, the fertilizer impacts on the humic substances composition have not been widely studied, and the researchers data is fragmented. The results demonstrated in Table 4.18. Analysis of groups and fractional composition of humic substances is made a number of chemical methods, the purpose of which transferring of humic particle into the soil solution, cleaning solutions from mineral and organo-mineral colloids, and finally humic acid division into certain groups and fractions for carbon quantification determination. However, the fertilizer impacts on the humic substances composition have not been widely studied, and the researchers data is fragmented. Due to the fact that humic substances are hypothesized to come from lignified tissues of plant detritus and lignin of grasses and cereals contains roughly equal fraction of guaiacyl propane, syringyl and p-hydroxyphenyl propane units (Kogel-Knabner, 2002), which suggest that impact on humus of residues of wheat and maize should be similar. In present study, the distribution of fulvic acid (FA) and humic acid (HA) and their fraction demonstrated in Table 4.18.

The results indicated no significant difference between all treatments, and the mean higher content of active FA total and its fraction was found 100 kg P₂O₅ ha⁻¹ dosage, however stable fraction of HA-2 was found in a less values of level application. The biggest results of stable HA-2 Calcium humate and HA total fractions were found in control plot for both depths, which may have indicated that fertilization along with biomass amount in plots affected HA and FA distribution. This is with accordance with Galantini and Rosell (2006), where long-term wheat cultivation and NP fertilization resulted in significant alteration in the soil organic fractions distributions. Fertilizer treatments increased labile organic fraction contents and modified the quantity and quality of the humified fraction and an increase of the plant residue inputs enhanced the labile SOM content and the less stable groups. Furthermore, the dynamic of SOC fractions may be depending on the annual precipitation and its seasonal distribution (Galantini and Rosell, 2006).

Table 4.18. Soil Humic substances distribution response to the different phosphorous fertilization levels under long term phosphorous fertilization after wheat rotation.

depth	P-level	Humic substances fractionation								
		FA1a	FA1	FA2	FA3	HA1	HA2	HA3	HA % total	FA % total
0-15cm	0	17.81±6.8	1.71±0.11	0.35±0.24	11.47±7.36	0.87±0.91	34.5±3.61	8.82±3.39	44.19±7.11	31.34±6.5
	50	28.38±12.8	2.36±2.1	0.81±1	5.71±4.25	0.22±0.23	31.29±3.93	13.52±2	45.03±5.3	37.26±8.91
	100	21.46±9.1	3.16±2.3	5.68±3.99	14.26±6.1	2.59±2.21	17.92±9.55	7.76±1.54	28.27±9.7	44.56±10.17
	200	22.98±5.3	1.32±0.16	0.95±0.6	11.17±2.99	1.64±1.29	31.57±2.7	8.95±4.29	42.17±6.86	36.42±5.4
F		ns	ns	ns	ns	ns	3.44	ns	ns	ns
P>f		0.07								
15-30cm	0	5.46±0.08	2.58±1.11	4.58±2.78	12.07±1.4	2.23±0.92	34.08±1.84	9.94±0.69	46.25±2.0	24.69±2.9
	50	21.68±8.25	1.22±1.02	1.1±1.46	13.67±5.76	4.89±5.82	23.85±15.21	6±1.43	34.74±19.5	37.67±3.14
	100	13.58±4.15	2.47±0.86	4.63±3.07	19.89±12.53	2.37±2.25	29.24±4.57	10.25±9.56	41.85±14.2	40.57±9.9
	200	9.44±2.8	1.62±0.83	9.46±5.48	13.86±8.45	3.53±2.33	28.69±4.53	8.62±2.78	40.85±4.2	34.39±8.7
P>f		ns	ns	ns	ns	ns	ns	ns	ns	ns

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹. FA1a – Fulvic acid fraction 1a-oxidant. FA1- Fulvic acid fraction 1, soluble. FA2- Fulvic acid fraction 2, bonded with Ca²⁺. FA3 - Fulvic acid fraction 3, bonded with clay particles. HA1- Humic acid fraction 1, Soluble. HA2- Humic acid fraction 2, bonded with Ca²⁺. HA3 - humic acid fraction 3, bonded with clay particles.

Generally, humic acid of carbonate soils almost completely represented by fraction 2 (Ca-related), which is proved by the control plots soil for both depths. In the 15-30 cm depth, the pattern of HS distribution is showing the maximum concentration of more stable HA in control samples (Table 4.17). Moreover, it can be notified, that 200 kg P_2O_5 ha⁻¹ dosage contains more balanced amount of all stable fractions and humic, which may be defined as positive process in 15-30 cm depth. However, all results were not significantly different among each other, that is in accordance to theory of stability of those substances, especially in a clay soil. Similarly, Simonetti *et al.* (2012) showed that after 44 year of mineral fertilization, the humic C concentration was not statistically different from untreated soil. Generally, arable grassland soils contain a more fulvic acids and the proportion of highly decomposed and microbially derived organic matter (Leinweber *et al.* 2001). Some studies reported that after 13 years of only maize cultivation 22% of SOC was turned over and after 50 years SOC associated with fine Clay fraction (FA-3, HA-3) was rapidly depleted under cultivation (Torres-Sallan *et al.* 2017). Compounds of humic acids with mobile Fe-Al oxides (HA-3) have an insoluble nature and resistant to microbiological impact (Popov, 2004). Humification is the prolonged stabilization of organic substances against biodegradation (Kögel-Knabner, 2002) and this indicate that more stable reaction is going on under control plots and 50 kg P_2O_5 ha⁻¹ dosage, which means the fertilization has some insignificant impact on the speeding microbiota's reactions and creation/dissolution more stable fraction (Wlash *et al.* 2012). Luo *et al.* (2015) results suggested that long-term soil P-deficiency can significantly decline soil microbial biomass, dehydrogenase activity and bacterial diversity.

It could be concluded that fertilizer application transforms organic carbon fraction distribution to more aliphatic (more fulvic), thus the SOC quality was affected by management, and even so changes in plant exudates and microbial response, climatic conditions should also be considered.

4.2.4.2. Soil Nitrogen Content

Soil nitrogen (N) content was not significantly affected by control, 50 and 100 fertilization 0.80 g N kg^{-1} among the treatments and with $200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ doses application slight increasing in up to 0.89 g N kg^{-1} in 0-15 cm soil depths (Table 4.17). In 15-30 cm depth N soil content significantly increased in $200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$. Soil N content in 0-15 cm depth demonstrated to have insignificant positive correlation with soil SOC content (Table 4.21). The C:N ratio was positively strongly correlated with SOC content in both soil depth. Ratio showed higher values for control and $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ treatments in 15-30 cm depth, than those treatments in upper horizon. The C:N ratio for 100 and $200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ demonstrated opposite trend. Compare to the maize growing season, can be noted that soil after wheat has lower C:N ratio in 15-30 cm depth, and higher in 0-15 cm depth. Soil N content was positively correlated with wheat shoot and root biomass (Table 4.21.) The C:N ratio for wheat growing season demonstrated much higher value than in maize growing season for upper horizon, which we can explained by slightly higher SOC content.

4.2.4.3. Soil Phosphorous Content

Under the wheat rotation period, the soil P-available content was expectedly significantly affected by fertilization in both soil depths, there minimum in control plots ($2.05 \text{ P mg kg}^{-1}$) a maximum values in $200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ dosage ($20.2 \text{ P mg kg}^{-1}$) in 0-15 cm soil depth (Table 4.17.). The soil P content was found to have positive strong correlation with permanganate oxidizable carbon in 0-15cm soil depth, and with soil nitrogen content in 15-30 cm soil depth, also with wheat shoot and root biomass (Table 4.21.). The C:P ratio of wheat growing season showed higher value (3582) under zero P application and decreasing down to 351 under $200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ dosage in upper horizon. Similar results were observed for 15-30 cm soil depth. Soil's both depth horizons's C: P ratio was negatively

correlated with wheat shoot biomass. The seasonal variability in the C:P ratio also can occur and indicated seasonal responses in biomass C and P (He *et al.* 1997).

4.2.4.4. Soil CO₂ Flux Under The Field Condition

From 2016 to 2017 regularly soil-atmosphere CO₂ exchange were measured with the static chamber method (Appendix 2.), which represents summary of organic carbon decay, root and microbial respiration, as well as in the litter decomposition and calcium carbonate (CaCO₃) dissolution. The soil flux during the wheat plant growth results presented in Table 4.19. All treatments including zero P application were compared also with bare soil without crop-cover (bare soil), laid near the experimental plots area. The impact of temperature rise on soil flux could be noticeable only at early spring phase, where slightly increasing of flux was appeared even on not cultivated bare soil. In all treatment, the soil flux under wheat growing season was noticeable higher compared to bare soil, exclude relatively low ambient temperature (12°C), there bare soil flux shows average 1.8 CO₂ flux $\mu\text{mol m}^{-2} \text{s}^{-1}$ and all treatments shows similar results average 2.5-2.7 CO₂ flux $\mu\text{mol m}^{-2} \text{s}^{-1}$. Since rising temperature up to 20°C and plants maturity up to 4 months, all treatments displayed increasing soil flux. Drastic difference can be noticed at early spring stage with increasing ambient temperature up to 26°C (Table 4.18.), where 100 kg P₂O₅ ha⁻¹ treatments reaches maximum 7.98 CO₂ flux $\mu\text{mol m}^{-2} \text{s}^{-1}$, control reaches 5.39 CO₂ flux $\mu\text{mol m}^{-2} \text{s}^{-1}$ and bare soil shows only 3.48 CO₂ flux $\mu\text{mol m}^{-2} \text{s}^{-1}$, meanwhile 50 and 200 kg P₂O₅ ha⁻¹ shows close results as 6.18 and 6.79 μmol respectively. This is in accordance to Mukhopadhyay *et al.* (2014) where at 24-h cycle of study the diurnal variation CO₂ concentration the mean flux of mowed grassland was 6.88 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at similar temperature 29.38±3.39°C. In decade-duration surface warming (1– 2°C) experiment was demonstrated no effect on soil respiration rates, while N addition had elevated CO₂ effects (Strong *et al.* 2017). Present study control plots through all plants maturity stage shows a smoother curve and varies within 4-5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ without sharp changes in independent of the rise and fall of temperature.

Table 4.19. Soil carbon dioxide flux response to different phosphor fertilization levels under wheat growing season.

CO ₂ flux $\mu\text{mol m}^{-2} \text{s}^{-1}$ under Wheat growing season 2017									
p-level	observation date								
	20feb	07mar	20mar	27mar	11apr	25apr	15may	Mean CO ₂ flux	
0	2.78±0.3	5.4±0.19	5.06±0.43	4.58±0.46	4.59±0.8	4.26±0.26	4.4±0.5	4.44±0.53	
50	2.84±0.52	6.18±0.8	4.97±1.06	5.57±0.55	3.88±0.53	5.18±0.72	4.55±1.13	4.74±0.84	
100	3.18±0.53	7.98±0.75	5.19±0.86	4.69±0.98	3.86±0.45	4.31±0.56	5.38±1.37	4.94±1.06	
200	3.02±0.09	6.79±2.04	4.6±0.72	4.34±1.37	3.68±0.6	4.85±0.9	3.9±0.66	4.45±0.82	
Bare soil	2.1±0.02	3.48±0.6	2.77±0.08	1.47±0.18	2.1±0.4	2.23±0.34	2.03±0.005	2.31±0.46	
T °C	NS	NS	NS	NS	NS	NS	NS	NS	
	12.9°	26.27°	20.78°	23.26°	25.68°	27.89°	33.13°		

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹. In the present table "bare" is the flux obtained from bare soil laying between the experimental plots without cover crop. The f-ratio and the p-value is calculated excluding the bare soil data.

Different values of CO₂ flux might be due to bigger percentage of root and microbial biomass. Similarly, Ortas *et al.* (2017), (unpublished TARP project data) measured CO₂ flux under long-term organic experiment in similar soil and territory, where they found similar results. No significant effect of P on CO₂ was also found by Huff *et al.* (2015), Wang *et al.* (2016) and Wenlong *et al.* (2017). Whereas, Song *et al.* (2011) reported that soil respiration showed a significant decline in the first year of P fertilizer application, moreover, P addition generally inhibited soil enzyme activities and reduced soil microbial biomass. Huff *et al.* (2015) indicated that lack of response of CO₂ to P-addition could be due to N-availability and changes in plant allocation to fine roots and root exudates with increased P-availability. It is important to take into account, that increased soil fluxes may demonstrate positive process occurring, that accompanying enhanced plant inputs, microbial activity play major role for promote and maintaining sufficient SOM levels (Sanderman *et al.* 2017; Lange *et al.* 2015).

Due to knowing the detail amount of biomass CO₂ uptake for all treatments plot in this study, and an accurate gas flux measurements comparison might be a good approach to investigate soil–plant–atmosphere gas-exchanges systems.

In present study, balances of CO₂ gain and losses per 3 months (growing period) demonstrated in Table 4.20.

Table 4.20. Total CO₂ uptake and efflux of experimental area under wheat 3 months cultivation period

Wheat growing season CO ₂ gas exchanges		
p-level	Total soil CO ₂ efflux	Total biomass CO ₂ uptake
t ha ⁻¹ growing period ⁻¹		
0	15.3±3.72	24.2 ±1.9
50	16.4±5.84	27.3 ±3.1
100	17.1±7.38	32.5 ±0.5
200	15.4±5.7	40.6 ±5.1
Bare soil	8.01±3.22	0.0

Calculated from mean data. 0: Control; 50: 50 kg P₂O₅ ha⁻¹; 100: 100 kg P₂O₅ ha⁻¹; 200: 200 kg P₂O₅ ha⁻¹

As it presented in Table 4.20, all treatments have positive impact on CO₂ balances, and significantly higher results than maize growing season, mostly due to lower soil fluxes and higher biomass. The highest fertilization dosage (200 kg P₂O₅ ha⁻¹) demonstrated the highest biomass CO₂ uptake; therefore, P fertilization has positive impact on biomass carbon sequestration.

Correlation test for wheat growing season result demonstrated in Table 4.21.

Table 4.21. Correlation test for both soil horizons, wheat growing season

0-15 cm										
	Soil P	SOC	Poxc	Soil N	Soil C:P	Soil C:N	Shoot	Root	Grain	CO ₂ flux
Soil P	1									
SOC	0.555	1								
Poxc	0.722**	0.474	1							
Soil N%	0.567	0.517	--	1						
Soil C:P	-0.757**	-0.809**	-0.739**	-0.755**	1					
Soil C:N	--	0.784**	--	--	--	1				
Shoot	0.640*	0.504	0.778**	0.607*	-0.770**	--	1			
Root	0.660*	0.772*	0.762*	0.60*	-0.79*	--	0.593*	1		
Grain	0.632*	--	--	--	--	--	--	--	1	
NAR	--	--	--	--	--	0.521	0.549	--	--	1
CO ₂ Flux	--	--	--	--	--	--	--	--	--	1
15-30 cm										
Soil P	1									
SOC	--	1								
Poxc	--	--	1							
Soil N%	0.779*	--	--	1						
Soil C:P	-0.794*	--	--	-0.605*	1					
Soil C:N	--	0.665*	--	--	--	1				
Shoot	0.512	--	--	0.783*	-0.632*	-0.588*	1			
Root	0.698*	--	--	0.644*	--	--	0.593*	1		
Grain	--	--	--	--	--	--	--	--	1	
NAR	--	--	--	--	--	--	0.549	--	--	1
CO ₂ Flux	--	--	--	--	--	--	--	--	--	1

PoXC – permanganate oxidizable carbon. NAR- plant's net photosynthetic effectiveness in assimilating CO₂.

**: p<0.01; *: p<0.05

5. CONCLUSION

The objectives of present study was to: (i) analyze the effect of long-term inorganic chemical P fertilization on the plant biomass carbon uptake and SOC pool for wider environmental protection, (ii) estimate effect of long-term P fertilization on CO₂ flux related with SOC accumulation.

The tested hypothesis was long-term field condition P-fertilization can affect Carbon Sequestration in soil and plant biomass and impacts on soil CO₂ flux.

Experiment was conducted on a long-term P fertilizer applied field experiment, which was setted up in 1998. During 2016 and 2017, wheat and maize successions of plant were grown. During those terms soil organic carbon, soil CO₂ flux and photosynthesis rates and plant carbon uptake were measured.

In the maize growing season, phosphorus (P) fertilization significantly increased maize shoot, root and total biomass production. The P fertilization did not affect plants tissues C content; however, significantly increased shoot's N and P concentration. Consequently, maize biomass's N uptake was significantly positively affected by P fertilization. Maize biomass's carbon uptake and CO₂ fixation demonstrated gradually increasing with P fertilizer dosages, however, with weak statistical significance. The P fertilization had no effect on Net Photosynthesis Assimilation Rate in maize plant.

The soil organic carbon (SOC) content results shows that solely P fertilization did not have negative nor positive impact on soil carbon storage after maize cultivation season. Despite the fact, that fertilization increased root biomass production, it did not embrace soil carbon storage in soil.

The soil CO₂ fluxes were not significantly affected by P fertilizer application. The all treatments demonstrated the similar results and it was concluded that the main reason of slight fluxes changes between maize growing stages were mostly affected by atmospheric temperature fluctuation. Comparative analysis demonstrated that all treatments has positive CO₂ exchanges balances per

growing season, where total biomass CO₂ uptake is higher than total soil CO₂ losses from experimental area per cultivation period. The highest P fertilization dosages (100, 200), demonstrated less CO₂ losses from soil efflux (24.3 and 22.5 t ha⁻¹ respectively) due to slightly lesser soil CO₂ flux and higher maize biomass CO₂ capturing (39.8 and 37.5 t ha⁻¹ respectively). Absence of P fertilization (control plot) demonstrated lower results then recommended fertilization dosage, biomass CO₂ capturing was 29.9 t ha⁻¹, and soil CO₂ losses from soil efflux was 24.3 t ha⁻¹, therefore, positive CO₂ balances.

It can be concluded, that P fertilization dosages demonstrated to have positive impact on CO₂ balances under the hottest summer for maize cultivation period under calcareous soils. Balanced fertilization has shown the beneficial effect on plant biomass production and consequently biomass improves CO₂ soil-atmosphere gas-exchange balances.

In the wheat growing season, P fertilization significantly increased wheat shoot, root, grain yield and total biomass production. The P fertilization did not affect wheat plant tissues carbon concentration, however significantly increased root's N and shoot's P concentration. Consequently, wheat biomass's nitrogen uptake was found to be statistically positively affected by P fertilization. Wheat biomass's carbon uptake and total CO₂ fixation were positively significantly affected and consistently increased with P fertilizer dosage applications increases. Wheat Net Photosynthesis Assimilation Rate did not demonstrated to be affected by P fertilization.

The result of soil after wheat rotation demonstrated significant positive impact of fertilizer on SOC content. Several separation and fractionation methods on the basis of the chemical characteristics of SOC have been performed in an attempt to select moderately stable and active pools of carbon. Humic substances fractionation demonstrated that fraction was insignificantly changed at higher dosages to more oxidized conditions compare to control with more stable fraction. This indicates that process of degradation is slightly higher in fertilized plots,

which may be caused by higher microbial activity and presence of easy oxidizable carbon.

Regarding to gas exchange, soil CO₂ fluxes were not significantly impacted by solely fertilization. The all fertilizer treatments demonstrated the similar results in between each other. However, fluxes were found to be lesser than in summer season. It was hypothesized that fluxes dynamics mostly related to soil temperature, plant maturity stage, its root respiration, exudates process and microbial activity. As previously was mentioned, fertilization considered may have indirect effect on CO₂ fluxes, as a nutrient provider for bigger root biomass and thus its respiration.

Comparative analysis demonstrated that all fertilizer dosages have positive impact on CO₂ balances, and significantly higher results than maize growing season, mostly due to lower soil fluxes and higher biomass carbon uptake. Control plot demonstrated lower results than fertilized plots, control biomass CO₂ capturing was 24.2 t ha⁻¹, and soil CO₂ losses from soil efflux was 15.3 t ha⁻¹. The highest fertilization dosage (200 kg P₂O₅ ha⁻¹) demonstrated the highest biomass CO₂ fixation (40.6 t ha⁻¹) and low soil efflux (15.4 t ha⁻¹); therefore, phosphorus fertilization has positive impact on biomass carbon uptake (sequestration).

In a comparison of CO₂ fluxes between not-cultivated bare soil and fertilized covered soil, it was found that bare soil have significantly lower fluxes, however, cover crop compensated it with the significant crops CO₂ fixation, which is greatly exceed the soil effluxes.

It can be concluded, that:

1. Phosphorus fertilization has positive impact on plant biomass of maize and wheat plants and, therefore, increasing atmospheric CO₂ capturing.
2. Phosphorus fertilization has positively affected SOC content in winter-season under wheat cultivation, but demonstrated no impact in summer-season under maize plant cultivation.

3. Phosphorus fertilization in all dosages did not demonstrate neither positive nor negative impact on soil CO₂ flux. However, in planted plots compare to bare soil there are higher values of CO₂ flux.
4. Phosphorus fertilization did not improve photosynthetic CO₂ assimilation characteristic for neither maize nor wheat plants.

This study requests a developing a balanced land use plan in order to encourage the process of carbon sequestration in soil during long-term fertilization. The results demonstrate that SOC storage should be assessed in terms of possible fractionation of total C stocks with respect to SOC structure and lability. Further detailed information of the fractions and stability of SOC pools are critical to understand the cause of different SOC degradation rate and balances.

Recommendation for future research.

The long-term analysis of organic carbon substances changes along with active fraction analysis could be beneficial to better understanding of carbon management under the Mediterranean climatic conditions and may have better aftermath on explanation of changes of carbon pools in agricultural fields. Moreover, in a concept of SOC sequestration a mechanisms of regulating nutrients release from plants residues and roots exudates need to be carefully investigated. The global carbon cycle is varying, as the fluctuation of seasonal CO₂ concentration, net assimilation rate and land use. Due to all possible influential factors, the chemistry of soil-atmosphere carbon exchange remains unclear and a challenge for future research. The work need to be continued with more detailed measurements on carbon fixation and carbon loses related with soil crop managements

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CURRICULUM VITAE

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APPENDICES

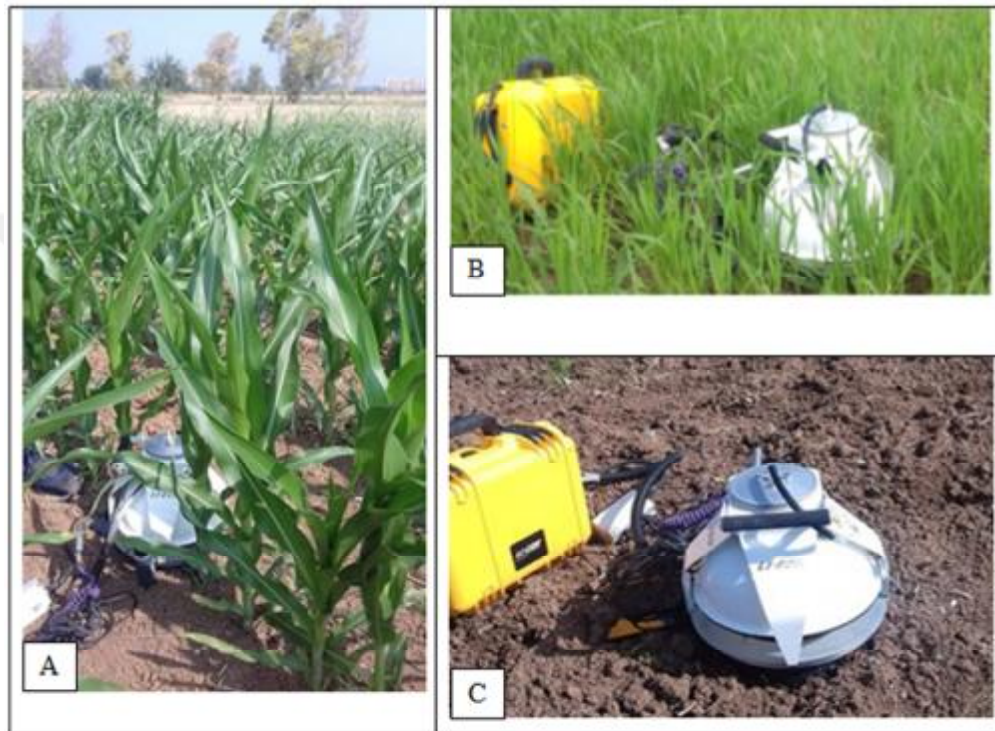


Appendix 1. Soil characteristics response to the different phosphorous fertilization levels under Maize-Wheat rotation, 2015-2016 observation year.

Maize rotation 2015									
depth	P-level	PoXC mg kg ⁻¹	SOC g kg ⁻¹	Pmg kg ⁻¹	N%	C:N	C:P		
0-15cm	0	599.2 ±71.9	7.00 ±0.29	6.07 ±1.8	0.112 ±0.07	6.2	1249.7		
	50	445.3 ±241.9	6.72 ±0.83	6.36 ±0.8	0.101 ±0.04	6.6	1090.6		
	100	603.1 ±22.5	7.53 ±0.6	7.41 ±0.1	0.098 ±0.03	7.7	1015.0		
	200	590.0 ±44.6	6.58 ±0.3	10.00 ±3.9	0.102 ±0.01	6.4	837.6		
	F	ns	ns	ns	ns				
ns									
15-30cm	0	389.9 ±201.9	5.88 ±0.1	2.90 ±0.7	0.098 ±0.01	6.0	2183.7		
	50	634.1 ±105.3	6.69 ±0.4	3.81 ±1.01	0.104 ±0.03	6.4	1868.0		
	100	632.0 ±52.4	6.14 ±0.3	4.10 ±0.5	0.090 ±0.03	6.8	1527.5		
	200	601.3 ±94.1	6.24 ±0.2	5.29 ±1.8	0.090 ±0.07	6.9	1387.2		
	F	ns	ns	ns	ns				
ns									
ns									
Wheat rotation 2016									
depth	P-level	PoXC mg kg ⁻¹	SOC g kg ⁻¹	Pmg kg ⁻¹	N%	C:N	C:P		
0-15cm	0	611.8 ±131	8.98 ±1	6.03 ±0.23	0.11 ±0.008	8.3	1489.2		
	50	474.3 ±123.6	8.44 ±0.45	7.75 ±0.3	0.11 ±0.023	7.4	1092.8		
	100	714.5 ±43.7	9.41 ±0.64	17.4 ±2.03	0.10 ±0.005	9.1	550.2		
	200	596.0 ±179	9.27 ±0.5	25.5 ±2.14	0.09 ±0.002	9.9	364.1		
	F	ns	ns	74.23	ns				
ns									
<0001									
15-30cm	0	368.8 ±167.6	7.73 ±0.35	3.96 ±0.69	0.10 ±0.021	8.0	2027.3		
	50	348.6 ±64.1	8.73 ±0.8	8.77 ±0.98	0.08 ±0.005	9.8	1015.0		
	100	581.7 ±62.3	6.70 ±2.48	8.31 ±2.09	0.09 ±0.006	7.3	804.8		
	200	684.1 ±221.6	9.44 ±0.7	15.88 ±5.2	0.08 ±0.009	10.9	650.7		
	F	ns	ns	5.90	ns				
ns									
0.02									

Mean of three replicates ±S.D. NS – not significant at $p < 0.05$ by LSD test. P0: Control; P50: 50 kg P₂O₅ ha⁻¹; P100: 100 kg P₂O₅ ha⁻¹; P200: 200 kg P₂O₅ ha⁻¹. PoXC – Permanganate oxidizable carbon

Appendix 2. Field CO₂ measurement by Automated Soil Gas Flux System portable chamber (LI-8100A), under maize growth season (A), under wheat growth season (B), bare soil flux measurement (C).



Photographs made by the author.