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Investigation of Soil Health and Sustainable Management in Rice-Based Production Systems

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بسم الله الرحمن الرحيم

I lovingly dedicate this thesis to my beloved parents,

Farahnaz and Reza,

who have been endlessly supporting me and giving me courage each step of my life and my sister Pantea, who I have always had near me

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"The LORD God formed the man from the soil of the ground and breathed into his nostrils the breath of life, and the man became a living being"

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Chapter one

General introduction

"THE SOIL WORKS FOR YOU IF YOU WORK FOR SOIL"

USDA-United State Department of Agriculture, 2010

Soils are remarkable materials, constituted of an extraordinarily diverse range of mineral and organic components. They are the interface between the atmosphere, biosphere and the subsurface zones, linking them to the hydrosphere, and provide the land surfaces which physically support all terrestrial biomass. Soils are highly heterogeneous in space and time with many different types, and concomitant properties, distributed across the planet (Brevik, 2013; Ritz and Van der Putten, 2012). The different combination of five soil factors, including parent material, topography, climate, organisms, and time results in the genesis of soils with various properties (Jenny, 1941)

Due to the growing world population, a principal goal of the present agricultural management systems is to sustain and/or increase productivity. From a social point of view, sustainability deals with food security[1] and food safety. Summarizing the concern, there is still the problem of how to feed the entire world population with enough and safe food (FAO, 2010; Godfray et al., 2010). The Food and Agriculture Organization of the UN (FAO) estimates that the world may need to increase food production by 60% compared to current levels of production (Alexandratos and Bruinsma, 2012). Instead, Pimentel (2006) stated that world cereal grain production per capita reduced steadily from the early 1980s through 2000.

[1] "A situation that exist when all people, at all times, have physical, social and economic accesses to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO, 2003).

According to Larson et al. (1991), sustainable agriculture should supply current production goals without compromising the future. It is believed that sustainable measures are those that enhance the environment, natural resources, and related dimensions of society. In agriculture, sustainable systems emphasize the sustainability of the soil resource that is, along with the other essential resources of water, air, and light maintaining our food production (Hatfield and Karlen, 1993). Soil quality in managed ecosystems is strongly influenced by management practices and land-use decisions. Unfortunately, past management of agriculture and other ecosystems has considerably degraded and diminished the quality of many soils throughout the world (Oldeman, 1994). In particular, the continuous production of row crops and mechanical cultivation has resulted in physical loss of soil, displacement through erosion, and large decreases in soil organic matter content with a concomitant release of CO2 to the atmosphere (Nacinovic et al., 2014; Ouyang., 2013; Houghton et al., 1983).

An important question is: what agricultural practices are sustainable? As described in Robertson and Harwood (2013), this is an area of intensive research. Sustainable practices must meet the three criteria determined in Elements of Sustainability: They must be economically viable, environmentally safe, and socially acceptable. There is no single prescription for sustainability; sustainable practices will alter by cropping pattern, local environment, and socioeconomic system. Nonetheless, emerging research results suggest that locally sustainable systems tend to be more resource conservative than less sustainable systems.

Each cropping practice must be evaluated in a whole-system context to adequately evaluate its contribution to a system's sustainability, for instance Snapp et al. (2005) reported that conservation tillage typically slows or stops soil organic matter loss and thus can be considered a resource-conserving, sustainable cropping practice. However, tillage controls weeds

in cropping systems, and in the absence of tillage weed control is typically achieved with herbicides, which have environmental and economic costs different from those of tillage. Most of the sustainable agricultural production research of the past two decades has focused on comparisons of crop rotations and use of cover crops and other systems component practices (Abdollahi et al., 2014; Franzluebbers et al., 2014; Robertson and Harwood, 2013).

Soil quality concepts are commonly used to evaluate sustainable land management in agroecosystems (Carter, 2002). In addition, it could be useful in determination of environmental quality (e.g. climate change) (Lal, 2011a; Pierzynski et al., 1994), and as a consequence of both, plant, animal, and human health (Barrios et al., 2012; Antunes et al., 2012). Hence, assessment of soil health has attracted a great deal of attention in recent years because of growing public interest in determining the effects of soil management practices on the physical, chemical, and biological soil properties and consequently on the soil quality relative to sustainability (Yao et al., 2013; Schoenholtz et al., 2000). Soil health and soil quality are basically the same idea, "soil health" more often used by farmers and land managers, while "soil quality" more often used by academic researchers (e.g.soil scientists, agronomists, and pedologists) (Magdoff and van Es, 2009). In this thesis these two terms are used synonymously.

The scientists attempt to interpret their scientific knowledge and information on soil function into practical tools and approaches by which land managers can estimate the sustainability of their management (Bouma, 1997).

Outline of the thesis

Two studies (the rice district in the Venetian territory (NE Italy) and paddy fields in the Philippines at IRRI[2]) that assess the relative impacts of various cropping patterns on indicators of soil quality and using indicator results to specify soil health condition are described in the four chapters of this thesis.

The first chapter gives an overview of the current literature pertaining to soil quality, soil quality indicators, and the effect of agricultural management on the soil indicators. Finally, specified research objectives are determined in this chapter. The second chapter describes the experimental set up of the studies and sites description. Chapter three reports the effect of various cropping patterns on soil physical, chemical and biological properties. Furthermore, assessment of total soil and plant elements in the study area is reported in this chapter. Additionally, chapter three describes soil health status of the study area using three soil quality indexing methods, namely, an additive index, a weighted additive index and systematic soil quality index were calculated by integrating indicator scores obtained either by expert opinion or principal component analyses. Finally, Chapter four consists of overall conclusions from this thesis.

[2] International rice research institute

1. Soil health assessment tools

"MANAGING FOR SOIL HEALTH MUST BEGIN

BY CHANGING THE WAY YOU THINK ABOUT SOIL"

USDA-United State Department of Agriculture, 2010

1.1 Soil quality

Soil quality has been described in several ways (Doran and Parkin, 1994; Larson and Pierce, 1991); for example, Johnston (1994) proposed that: "soil quality is a measure of the condition of the soil relative to the requirements of one or more societies and/or to any human needs or purposes". Although soil quality cannot be readily defined because it depends on multi-dimensional factors such as soil management practices, land use, and ecosystem and environment interactions, an expanded and complete version of soil quality definition has been proposed by Karlen et al., (1994) and the committee for Soil Science Society of America as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation". Further, Andrews et al. (2004) noted that soil quality of a particular soil is related to inherent capabilities, the intended land use and the management goals.

Soil quality can be investigated taking into consideration two different points of view: the inherent soil quality which is related to a soil's natural composition and its chemical, physical and biological properties that are influenced by the factors and processes of soil formation, in the absence of human impacts (Karlen et al., 2003). In contrast, dynamic soil quality is defined as the properties of soil that change due to the effect of human use and management system over time (USDA-NRCS, htt p:// soils.usda.gov).

The concept of soil quality is similar to that of human health (Magdoff, 2001) and is determined by keeping at an optimum level key soil properties and processes. Human health and global food security are linked to soil health, since human health is basically dependent upon plants, as they obtain elements from the soil and supply most of our nutrients through the food chain (Antunes et al., 2012). In other words, degraded soils reduce crop yields and produce crops with poor nutritional value, leading to malnutrition in the people who depend on those soils to produce their food.

Many studies have been doing in order to establish a balance between production rates and improve the quality of land resources. Hence, productivity should not be taken into account as the only goal of management systems, inferring that the emphasizing productivity may have contributed to soil degradation in the past (Griffiths et al., 2010; Govaerts et al., 2006; Larson and Pierce, 1991). Indeed, approximately 5 billion hectares (ha) of land are currently considered degraded; this represents about 43% of the vegetated land on earth (Brady and Wheil, 2008). Soil degradation is manifested in several changes to the soil, including loss of organic matter, loss of soil nutrients, reduced cation exchange capacity, lowered water-holding capacity, and loss of soil structure. Management goals are frequently individualistic, primarily focused on on-farm effects but can also be societal, including the broader environmental effects of farm management decision such as high rates of soil erosion, losses of organic matter, reduction in fertility and productivity, climate change, agrochemical contamination of soil and water, subsidy imbalance (e.g. over-use of fossil fuels or agrochemicals) (Andrews et al., 2004; Andrews et al., 2002b, Karlen et al., 2001). It is noteworthy to point out that the soil qualities (functions, properties) critical for each intended management goal are different even within the same field or under the same crop (Andrews et al., 2004).

The huge variety of studies have been carried on soil quality evaluation under various conditions including the effect of crop rotation (Abdollahi et al., 2014; Franzluebbers et al., 2014; Tein et al., 2014; Aziz et al., 2011), residue management (Imaz et al., 2010; Sharma et al., 2005), organic and conventional farming (Fließbach et al., 2007), mining areas (Afrifa et al., 2011; Shukla et al., 2004). However, there is no direct research concerning the effect of rotation systems on the soil quality of paddy fields.

Soil quality evaluation has been done using a wide variety of indexing techniques so far; although using a universally accepted method of assessment soil quality would assist scientists, land managers, and policy makers to understand soil quality status of different agricultural systems, no single method for assessing soil quality has been widely accepted due to the high complication of soil systems (Qi et al., 2009; Glover et al., 2000). These various methods include soil quality index (SQI) methods (Ferrarini et al., 2014; Andrews et al., 2002a; Doran and Parkin, 1994), Nemro quality index (NQI) method (Qi et al., 2009), fuzzy modeling (Xue et al., 2010; Torbert et al., 2008), soil quality card design and test kit (Ditzler and Tugel, 2002), the dynamic variation of soil quality models (Larson and Pierce, 1994), and the soil management assessment framework (Wienhold et al., 2009; Karlen et al., 2008; Masto et al., 2008; Andrews et al., 2004). Among these techniques, soil quality indices are perhaps the most commonly used methods today (Andrews et al., 2002a,b), owing to their flexibility and being user-friendly.

Science-based soil quality indices were innovated and developed due to the need for multi-objective decision tools for land managers, who make management decision, to evaluate management changes. Moreover, sustainable agroecosystem managements need evaluation of economic, social as well as environmental objectives (Andrews and Carroll, 2001). Soil quality indices apply concepts of soil ecology to evaluate the sustainability of soil ecosystem

management by effectively incorporating a variety of information for multi-objective analysis (Herrick, 2000).

1.2 Soil indicators

Assessment of soil management systems on soil functioning is possible through the study and evaluation of varieties in soil quality indicators. As described in Andrews et al. (2002a) soil quality indicators should be selected according to the soil function of interest, those which have the greatest sensitivity to changes in soil function, and the defined management goals for the system. In other words, the different soil quality indicators can represent different ecological functions. Several important soil functions related to crop production are suggested: adsorption and infiltration of water, retention and cycling of nutrients, pest and weed suppression, detoxification of harmful chemicals, sequestering of carbon and production of food and fiber (Gugino et al., 2007). In addition, the following five criteria were proposed in determining proper soil quality indicators: (1) sensitivity to variations in management practices, climate and human factor (2) well correlated with beneficial soil functions, (3) useful for elucidating ecosystem processes, (4) comprehensible and useful to land managers (be accessible to many users), (5) easy and inexpensive to measure (Bone et al., 2010; Doran and Parkin, 1996). The assessment of soil quality seems to be the best method to evaluate the functionality of the soil, describing the interactions that take place between the process and the different soil components (Fig. 1). Soil quality cannot be measured directly; it must be derived from a wide range of soil properties (physical, chemical and biological) that influence the capacity of soil to perform a function. Therefore, a group of indicators (minimum data sets), must be used in order to indirectly measure soil function of interest (Karlen et al., 2003; Doran and Parkin, 1994).

In literatures, various methods have been used for selecting a minimum data set to determine soil's quality, such as discriminate analysis (Lima et al., 2013; Xu et al., 2006), factor analysis (Rezaei et al., 2006; Shukla et al., 2006), pedotransfer functions (Matula and Špongrová, 2007), scoring function (Yang et al., 2010) regression equations (Masto et al., 2008), expert opinion (Glover et al., 2000; Andrews et al., 2002a; Andrews et al., 2004) and principal components analysis (Rahmanipour et al., 2014; Liu et al., 2014; Chen et al., 2013).

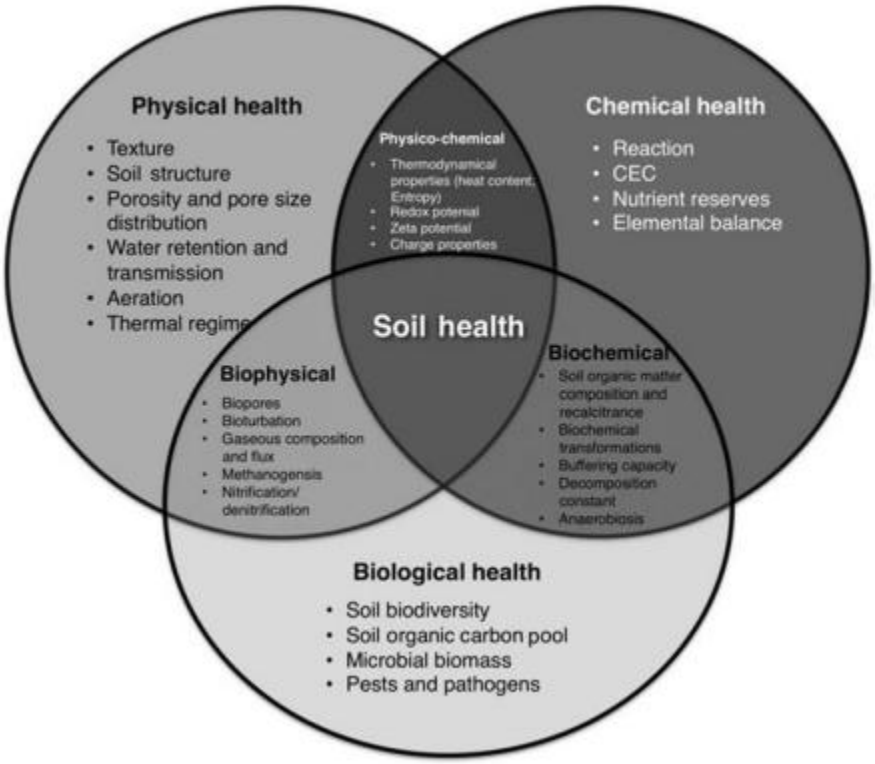


Fig. 1. Soil health components and the dynamic interactions between them (Lal, 2011c)

1.3 Some measured indicators for evaluating soil quality

1.3.1. Aggregate stability

Aggregates are defined as secondary particles that formed by combining soil primary particles (i.e. sand, silt, caly) along with cement agents, organic and inorganic materials (Bronick and Lal, 2005).

The aggregate stability is described as an indicator of the resistance against the complex external damaging functions such as rainfall, runoff, wind, surface seal or crust formation , compaction leading to decreased infiltration and subsoil aeration (e.g. machinery wheels), and as a general soil quality indicator (Angers et al., 2008; Kodesova et al., 2008; Doran and Parkin 1994).

In fact, the complex interactions among biological, chemical and physical processes in the soil affect soil aggregate stability. Factors changing aggregate stability were grouped as biotic (soil organic matter, activities of plant roots, soil fauna, and microorganisms), abiotic (clay minerals, sesquioxides, exchangeable cations) and environmental (soil temperature and moisture) (Chen et al., 1998). However, Soil particle size distribution is related to water retention, erosion processes, porosity and other soil properties that can change crop growth and productivity (Dìaz-Zorita et al., 2007).

Cropping pattern is believed to play an important role in aggregate stability and soil fragment size distribution. Many studies have indicated that, compared to continuous monoculture, crop rotations improve soil structure (Peixoto et al., 2006; Bronick and lal, 2005; Martens, 2000). Effectively, incorporating legumes in the rotation system improve aggregate stability (Raimbault and Vayn, 1991). However, many factors, including crop species, productivity, root physiology and function, residue management, can have influence on soil structure (Jozefaciuk and Czacho, 2014; Bronick and Lal, 2005; Franzluebbers, 2002; Chan and Heenan, 1996).

Soil organic matter as a cementing agent is one of the most important factor in formation and stability of soil aggregates (Spaccini and Piccolo, 2013; Saha et al., 2011; Lal, 2011b; Bronick and Lal, 2005).

There is a general agreement, over many years, that organic residues applied to soil with different management systems improved soil structure. The variation of the soil structure depends on the total amount of organic carbon, and also it relates to the chemical composition of organic matter and decomposition rate of crop residues. However, soil organic matter is considered as a good example of an ecosystem resource that is easily reduced without effective management. Several factors have been suggested to be responsible for the soil instability following soybean production; for instance it has been reported that soybeans have deleterious effects on soil structure due to limited return of biomass to the soil and residue biochemistry, including low concentration of phenols, although corn residues have high phenol concentration, high SOC and carbohydrate concentrations, which resulted in an increase of water stable aggregate formation (Martens, 2000). Furthermore Armbrust et al. (1982) stated that in a three-year comparison of soybean, sorghum and wheat, soybean plots had the smallest aggregate size and the least stable aggregates. Hamidpour et al. (2012) stated that addition of organic waste improves aggregate stability and the organic carbon content.

Kandiah (1976) stated that the optimum amount of organic matter to form stable aggregates is 4%, whereas Boix-Fayos et al. (2001) indicated macroaggregates stability increase when organic matter content is more that 5-6%. Green et al. (2007) showed that mean weight diameter of water stable aggregates is related to SOC and total N.

1.3.2. Trace elements

Environmental contamination involving potentially toxic elements (such as heavy metals) has attracted a great deal of attention in recent years, driven especially by concerns for human health (Wahsha et al., 2014a; Morgan, 2013). Heavy metal contamination of soils became a severe issue in agricultural production around the world in the past few decades. Many sources, of both natural and anthropic origin, can contribute to this contamination (Fanrong et al., 2011; Ramadan and Al-Ashkar, 2007; Kuo et al., 2006). Possible "natural" accumulation may be related to heavy metal-bearing rocks (e.g. As in sedimentary rocks: Bhattacharya et al. 2002; Sr in carbonate sediments: Kabata-Pendias and Mukhrjee, 2007; Ni and Cr in serpentine soils: Gonnelli and Renella, 2013). Anthropic activities such as agriculture, mining or industry make large use of metals and metal-enriched materials (e.g. Cd in plastic stabilizers and metallurgy, Cr in textiles, magnetic tapes, varnish and leather factories, Ni in batteries: Adriano, 2001; Siegal, 2002; Bini et al., 2008), and can be non-point sources of metals, including chemical fertilizers and pesticides, farm manure, sewage sludge, and wastewater irrigation.

Recognition of the sources responsible for soil contamination is an important issue, since high loads of heavy metals applied to soils, or stored in soils, may determine soil quality degradation, surface and groundwater pollution, and accumulation in plants, phytotoxicity and successive translocation to the food chain (Wahsha et al., 2012b; Bini, 2008). High concentration of toxic elements in soils would increase the potential uptake of these metals in the edible parts of vegetative tissues that may result in a direct pathway into the human food chain (Tariq and Rashid, 2013; Fanrong et al, 2011). Hence, in order to produce safe crops, it is essential to assess possible accumulation of harmful elements in agricultural land that could be helpful for proper application of pesticides, herbicides, organic and inorganic fertilizers, avoiding any human health

concern.

Plants display a different ability to absorb and translocate metals from soil to the aerial parts. Baker and Walker (1990) classified plants into three groups according to their ability to accumulate/exclude metals into their tissues: *Accumulators* are plants that can concentrate metals in their above-ground tissues to levels that exceed those in the soil; *Indicators*, are plants that concentrate metals in their above-ground tissues and the metal levels in the tissues of these plants commonly reflect those of the soil; *Excluders* are plants which effectively limit the amount of metal translocation from roots to shoots, (i.e. they maintain relatively low levels of metal in their shoots).

Rice is a widely diffused food crop in the world; it is consumed by more than three billion of the world's population (International Rice Research Institute Las Banas IRRI, 2005). It is one of the most economically important cereal crops in Italy, which is the largest rice producer in the European Union (EU); rice is cultivated in France (18,700 hectares) in Grecia (20,000 hectars), in Portugal (23,000 hectares), and in Spain (114.300 hectares), although approximately 2/3 of the European rice is produced in Italy (data from www.**enterisi**.it/). Rice cultivation in Italy is highly specialized and represents 70-80% of the rice farming surface, although in the last years soybean and maize have been successfully and increasingly grown as annual rotation crops in rice fields (Chisci, 2009; Russo and Callegarin, 1997).

Piedmont, Lombardy, Veneto, Emilia Romagna, Tuscany, Calabria and Sardinia regions are rice producers in Italy; according to the domestic market Piedmont with 120,000 hectares is a leader in rice production, followed by Lombardy which produces 6000 tons and the Veneto region which is characterized by the production of Vialone Nano Veronese IGP. In Veneto

approximately 3,000 hectares are used for rice production, even though 1,700 hectares of them are applied to cultivate rice in the province of Verona.

Rice cultivation is carried out on paddy soils that are managed in a special way; these soils are kept submerged seasonally, so that during the period of submergence, the soil undergoes reductive conditions. Various metals such as iron, manganese, silica, and phosphate become more soluble, disperse to the soil surface and move by diffusion and mass flow to the roots and subsoil. Zheng and Zhang (2011) stated that in paddy soils, the metal speciation probably undergoes changes among various moisture regimes; these include: (1) moist, oxidative conditions at a certain field capacity in land preparation step; (2) waterlogged conditions at seedling; (3) a short period of saturation (the establishment of temporary low oxygen conditions) during growing season, followed by draining and drying the surface soil at harvest (oxidizing conditions). These conditions and oxygen leakage by rice roots lead to the development of certain features (e.g. depletion pedofeatures), (Bullock et al., 1985), mostly occurring in rice fields, which are related to element redox-status variation, and hence affect their mobility and bioavailability.

1.3.3. Soil enzyme activities

Nutrient cycling in soil "involves biochemical, chemical and physicochemical reactions with biochemical processes being mediated by microorganisms, plant root and animals. The biochemical properties are more sensitive to environmental stress, and often play a major role in degradation and provide rapid and accurate information on soil quality" (Tabatabai, 1994). Biochemical parameters include microbial activity and soil enzymes involved in C, N, P and S cycles in soil (Gil Sotres et al., 2005); therefore, soil enzyme activities are used as soil quality and fertility indicators (Stott et al., 2010; Bastida et al., 2008; Trasar-Cepeda et al., 2008).

Indeed, soil enzymes have the potential to reflect the effects of environmental changes caused by natural or anthropogenic origin (e.g. land waterlogging, different crop rotation, land use changes, application of pesticides, herbicides, organic and inorganic fertilizers, tillage) on soil quality. Moreover, their activity measurement is rapid, easy and inexpensive (Raiesi and Beheshti., 2014; Aon et al., 2001).

Enzymes (i.e. β-glucosidase, arylsulfatase, acid phosphatase, chitinase) can be released from root plants, microorganisms, animals, organic compounds and soils (Makoi and Ndakidemi, 2008).The activity of soil enzymes is influenced by the nature, age and vegetation composition, management practices (e.g. application of fertilizers and manure), soil pH, SOM, soil moisture content, heavy metal pollution, temperature and microbial biomass (Singh and Ghoshal, 2013; Bastida et al., 2012; Song et al., 2012; Singaram and Kamalakumari, 2000; Doran and Parkin, 1994). Numerous studies, in particular, have found positive correlations between soil enzymes activities and organic matter content (Liang et al., 2014; Bastida et al., 2012; Wang et al., 2011). The soil enzyme activities occur owing to the presence of both extracellular and intracellular enzymes. Extracellular enzymes are biological catalysts exuded by both root plants and soil microorganisms; they may be bound on clay and humic particles and can be protected against decomposition, therefore keeping their activity even in the absence of soil microorganisms or under unfavorable environmental conditions for soil microorganism's activity (McDaniel et al., 2013; Nannipieri et al., 2002). Many studies have indicated that enzyme persistence in the soil ranges from a few days to several years depending on the location and soil conditions (i.e. temperature, pH, soil texture, soil organic matter, and depth) (Liang et al., 2014; Singh and Ghoshal, 2013; Singh et al., 2007; Bastida et al., 2006; Ekenler and Tabatabai, 2003).

Nutrient availability also influences the enzyme activity as various microorganisms control their enzyme production in response to nutrient availability (Chrost, 1991); therefore, soil enzyme activities could be varied due to using fertilizers, pesticides and herbicides, which are widely used to enhance soil fertility and crop yield (Ai, 2012; Zhong et al., 2010).

It is generally agreed that cereals (e.g. rice and maize) cropped with alternating leguminous crops (e.g. pea, soybean and alfalfa) improve soil enzyme activity due to enhancing soil microbial diversity and number. Zhang et al., (2008) reported that soil enzyme activity under the Soybean-Maize rotation, was higher than the continuous soybean cropping and soybeans-sorghum rotation systems. Liu et al. (2007) found that nutrient availability, rhizosphere soil microbes and thus enzyme activity in maize-soybean rotation system were higher than those of the corresponding monoculture crop; whereas the pot experiments of Yan et al., (2012) showed that there is no significant difference in urease activity among different cropping patterns of gramineae and leguminous (i.e. soybean-soybean, soybean-maize, soybean-mixed, maize-soybean, maize-maize and maize-mixed).

ß-glucosidase (βG) activity is considered as a soil quality indicator, since it has been shown to be sensitive to changes in soil and residue management. Its activity is related to the following soil functions: nutrient cycling, biodiversity and habitat, filtering and buffering, due to its importance in C cycling and providing simple sugars to support a diverse microbial population (Stott et al, 2010). Generally, increasing soil microbial biomass leads to increasing BG activity, which reflects soil's ability to break down plant residues and improve nutrients availability for subsequent crops (Stott et al., 2010). The C content of a soil is significantly correlated to β-glucosidase activity; it is a predominant enzyme in soils, since βG enzyme (EC 3.2.1.21) plays a major role in the degradation of soil organic matter and plant residues (Jinlong

et al., 2010). It catalyzes the hydrolysis of ß-D-glucopyranosides in the degradation of cellulose, providing simple sugars for the soil microbial population (Stott et al., 2010; Makoi and Ndakidemi, 2008).

Chitinase is considered as key enzyme to degrade chitine. It is produced by plants and micro-organisms. Chitinase release in soil is believed to be due to the microbial infections in plants as a defense of plants against pathogen infections. (Boiler et al., 1983).

Leucine aminopeptidases constitute a group of diverse exopeptidases that catalyze the hydrolysis of leucine residues from the amino-terminus of proteins or peptide substrates. This enzyme has variable temperature and pH optima and divalent cation requirements (Matsui et al., 2006).

Phosphatases are believed to play key roles in P cycles. In addition they are good indicators of potential mineralization of soil organic phosphorus and soil biological activities (Dick and and Tabatabai, 1984). Phosphatases activities depend on soil moisture content, vegetation composition, soil temperature and crop management practices.

Arylsulphatases are important enzymes responsible for providing inorganic sulphate ($SO4^{2-}$) from organic materials for plant nutrition; they catalyse the hydrolysis of aromatic sulphate esters ($R\text{-}O\text{-}SO3^{-}$) to phenols (R-OH) and sulphate ($SO4^{2-}$) (Dodgson et al., 1982).

Despite the large number of studies dealing with the soil enzymes in arable and forest soils (Yin et al., 2014; Wallenius et al., 2011; Kotroczó et al., 2011; An et al., 2008) there is still a lack of knowledge concerning the changes in soil enzyme activity under different alternate cropping systems over growth stages, in flooded soils. Flooded soils are predominantly anaerobic and differ from non flooded soils in several physical, chemical and biological properties. Therefore the presence of reduction and oxidation (redox) reactions leads to temporal and spatial

(vertical, horizontal) variations, affecting soil properties (e.g. the enzyme activities, the dynamics of organic and mineral soil constituents) (Kögel-Knabner et al., 2010; Cheng et al., 2009).

Enzymes of paddy ecosystems are important for improving and maintaining soil fertility to ensure rice productivity. Their activities are sensitive to soil disturbances (Mandal et al., 2007; Bohme et al., 2005).

1.4. Research objectives

The objectives of this thesis were:

- to examine the soil properties variability throughout the rice growing season;
- to assess the effect of different management systems on soil Quality, with comparison among different rotation systems in Italy and in the Philippines;
- to compare methods for choosing a minimum data set (MDS), namely, expert opinion (EO-MDS) versus Principal Component Analysis (PCA- MDS), and two methods of transforming indicators into unitless scores (namely, non-linear scoring method against linear scoring method);
- to compare different soil quality index outcomes, namely, an additive index, a weighted additive index and a systematic soil quality index, by integrating indicators.
- to determine the background levels of macro- and microelements in the study area, and possible contamination of soils and plants;
- to calculate the Translocation Factor (TF) of metals from soil to plant, and the possible hazard for human health.

Chapter two

Materials and methods

2.1 site description

As mentioned before the experiments were in two sections, Italy (Veneto region) and the Philippines (International Rice Research Institute).

2.1.1. Italy (Veneto region)

A part of this research was carried out in an agricultural land of Isola della Scala municipality, (45° 16´northern latitude and 11° 00´eastern longitude), which is located about 90 km west of Venice, and about 20 km southeast of Verona province, Veneto region, northern Italy (Fig. 2). The study area has a temperate submiditeranean climate; the annual main temperature and rainfall are 13°C and 856 mm, respectively. From the geological point of view, the area is connected with the formation of the Po river plain following the geodynamic evolution by uplifting of the alpine range. The river Tartaro also, which covers the north-western fringes of the town, affects the geology of the study area through the sedimentation and erosion phases.

The major geomorphological units that be found in the plain of Verona are as follows:

- The old alluvial fan of the Adige River with traces of very large braided channels
- The floodplain of the Adige river that has affected the alluvial fan digging fluvial escarpments
- The recent alluvial plain of the Adige, and some of the minor waterways.

In particular, the municipality of Isola della Scala is located in a very wide transition band where gravelly alluvial deposits of the old Adige change gradually to finer deposits. The major soil groups in the study area are sandy loam, calcareous Cambisols and Regosols, based on the soil

classification from World Reference Base for Soil Resources (WRB, 2010) and Sandy loam, mixed, mesic, calcareous, Antraquic Eutrudept based on USDA (2010).

2.1.1.1. Land management

The crop rotations in the study area included continuous rice (*Oryza sativa* L.) – rice – rice/ soybean (*Glycine max* L.) – rice – rice / fallow- rice/ and pea (*Pisum sativum* L.) – soya – rice.

Rice (*Oryza sativa* L.) is originally from the monsoon area of south-east Asia. It is a marsh species, even though different genotypes have been developed to grow on non-flooded soils. Owing to the presence of many cultivars, rice can adapt to very different environments, but it always needs high temperature.

Three rice (*Oryza sativa* L.) varieties: Vialone Nano Veronese, (which refers only to rice obtained from seeds rigorously selected from the Japonica species of the Vialone Nano variety) (www.risovialonenanoveronese.it), Carnaroli, and Arborio were used and planted in open field with different crop rotation systems.

The growing period of rice plant (*Oryza sativa* L.) is from May to October in the study area. The main soil tillage is a shallow ploughing (20-25 cm) using a frame plough at the beginning of spring. The seeds are germinated in the wide fields and raised under flooding conditions. The soil is kept in a distinct cycle of flooded and non-flooded conditions during the entire growing season. The total amounts of 170 kg N ha^{-1}as ammonium nitrate, 150 kg P ha^{-1}as phosphorus pentoxide, and 200 kg K ha^{-1}as potassium oxide were applied; pesticides and herbicides are also used when needed. The basal fertilizers were broadcast after transplanting at the rates of 17.47% of total N, 40% of total K and all of P during the growth, 57.28% of total N was top dressed at mid-tillering, 25.24% of total N and 60% of total K at early spike differentiation, respectively.

Fig. 2. Location of the studied area in Italy.

2.1.2. The Philippines (IRRI, Manila)

Another part of field and laboratory experiments were conducted at the IRRI[3] research farm in Los Baños, Philippines (14° 11′ N, 121° 15′ E; 21 m ASL), an irrigated lowland site, which is located about 50 km south east of Manila (Fig. 3). The station is located in the warm humid tropics with annual means of 2027 mm rainfall, 16.1 MJ m^{-2} d^{-1} solar radiations, and 26.8 °C temperature. The soil at the experimental site was classified as Aquandic Epiaqualf or Haplic Umbrisols according to (Soil Survey Staff, 1994) and (FAO, 2006) respectively.

2.2. Soil sampling

2.2.1. Italy

[3] The International Rice Research Institute (Manila, Philippines).

Surface soil samples (0 – 15 cm depth) were taken with a spade from four different cropping patterns with three replications, and four sampling times: in April (after dry field preparation), June (after seedling, waterlogged soil condition), August (after tillering stage of rice, late waterlogged) and October (after rice harvesting, drained soil condition) over the 2012 growing season. Each soil sample was a composite of sub-samples taken from 3 points within 350 m^2 of agricultural land. The soil samples were mixed well and bulked in the plastic bags for the analysis of chemical, physical and biological properties. In addition, we collected three undisturbed soil cores from each rotation system for those analysis requiring intact soil core samples. Part of the soil samples were refrigerated at – 10 $^{\circ}$C for biochemical analysis (i.e. Soil respiration (SR), potentially mineralizable Nitrogen (PMN), and Active carbon (AC). The soil samples were then air-dried at room temperature for 10-15 days, pulverized and sieved through a stainless steel sieve of 2 mm mesh diameter.

2.2.2. The Philippines: Manila, IRRI under ICON project

ICON experiment is a collaboration between IRRI and several German universities. It was established in December 2011, and the same treatments will be continued by 2015 (before starting the ICON trials, there was rice in all plots for several seasons) (Fig. 4).

We chose the following 3 different cropping systems and four Fertilizer treatments during two seasons (i.e. dry and wet seasons) so as to assess the effect of these management systems on soil quality under the study area:

Cropping systems:

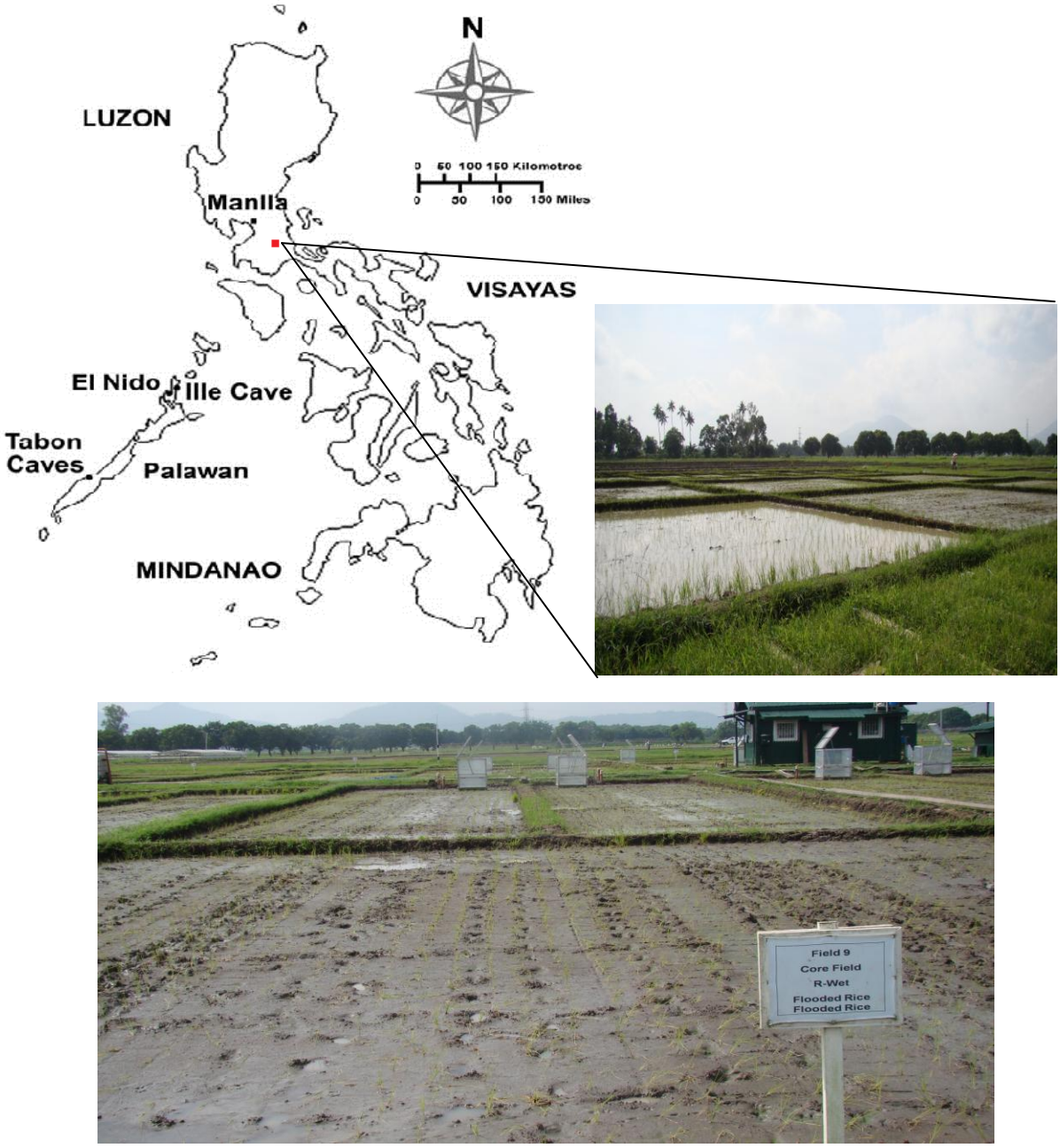


Fig. 3. Location of the studied area in the Philippines (IRRI)

- Flooded rice – Non-flooded rice (Water saving system; FR-NF)
- Flooded rice – Flooded rice (Conventional system; R-R)
- Flooded rice – Maize (Diversified system; R-M)

Fertilizer treatment:

- Conventional N management, no rice residues
- Zero N, no rice residues
- Conventional N management, with rice residues
- Zero N, with rice residues

The experimental layout of the study area was the randomized complete block design with tree replications, resulting in 36 sub-plots of 15 x 6 m isolated by embankments of about 0.3 m height.

The drained soil samples have been taken in dry and wet season after field preparation in December, 2011 and June 2013 respectively (30 samples for each season), Each soil sample was a composite of sub-samples taken from 3 points within 300 m^2 of each experimental design (Fig. 5).

We therefore had 3 cropping systems x 3 N/residue managements x two-factor experiment (dry and wet season) x 3 replicates = 72 soil samples (only 60 soil samples were taken due to the lack of conventional N management, with rice residues and zero N, with rice residues treatments for the Flooded rice – Flooded rice cropping system).

A total 60 soil samples well mixed, 2 mm sieved, and air dried were analyzed at International Rice Research Institute division of crop and environment science analytical laboratory. The samples were analyzed for soil pH, electrical conductivity (1: 5) (Violante and Adamo, 2000), soil respiration (SR) (MIPAF, 2000), potentially mineralizable N (PMN) (Gugino et al., 2007), active C (Weil et al., 2003), biological quality of soil index: micro-arthropod method (QBS-ar) (only for wet season, due to the lack of accesses to soil samples at a given time), aggregate size stability distribution (Ma´rquez et al., 2004).

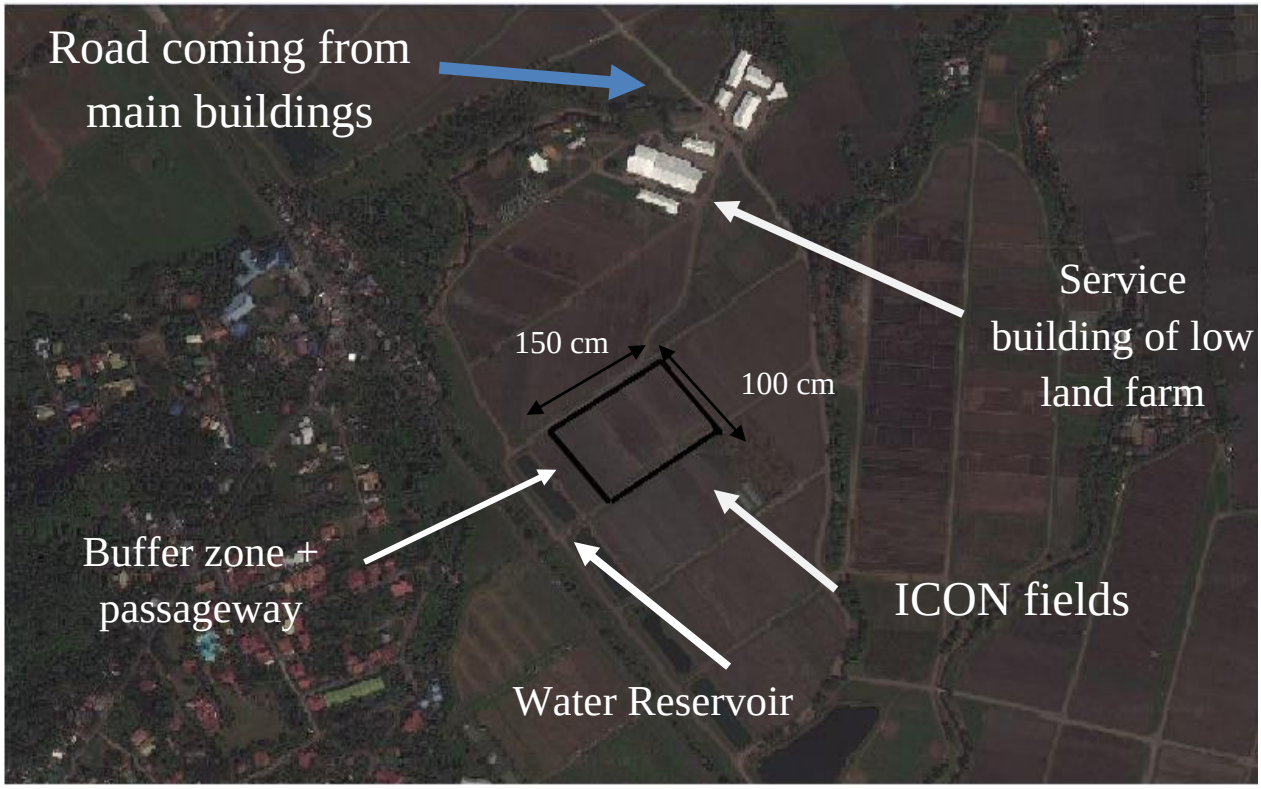


Fig. 4. Location of the ICON field experiment on the IRRI lowland farm

Fig. 5. Soil sampling at (IRRI, Manila)

Since not all the data set was available, we applied only some measured soil parameters (soil respiration, potentially mineralizable N, active C, aggregate stability, EC, pH, QBS) in

order to assess soil quality. The analytical procedures were the same than those applied in the assay in Italy, and reported in the above pages.

2.3. Plant sampling (Italy)

At early flowering and maturity (about 150 d after transplanting) plants were randomly sampled. The sampled plants were rinsed in tap water and distilled water to remove the soil residues, then separated into root, leaf, stem, and grains, oven-dried at 70 °C (for two days) to constant weight and manually milled in an agate mill into powder (< 100 μm) for elemental content determination (Fig. 6)

Fig. 6. The separated organs of rice into root, leaf, stem, and grains.

2.4. Laboratory analyses

Well mixed, 2mm sieved and air-dried samples were analyzed for most physical, chemical and biological properties at the University of Ca' Foscari Division of Environmental Science Analytical Laboratory. Routine soil analysis were determined according to the Italian soil analyses manual (2000, edition) which is published by the Ministry of Agriculture and Forestry.

2.4.1. Physical indicators

2.4.1.1. Soil particle size distribution

The soil texture was determined using the pipette method by Mecella and Scandella (2000) which corresponds to the international standard ISO 11277. In this method the coarser fractions are measured by sieving, and the finer from sedimentation rates based on Stokes' law. This law states that the amount that a particle sinks depends upon the density of the particle, i.e., denser (larger, usually) particles sink more than less dense (smaller) particles when suspended in a liquid.

The proportion of the values obtained from each fraction (sand, silt, and clay) was used to determine the soil texture class using the diagram in Fig. 7, according to the USDA classification.

The percentage of particle size fractions was calculated using the following formulas:

% Clay (< 0.002) = ((A x 50) – B) x (100 / C)

% Fine silt (0.02 – 0.002) = (((D+A x 50) – B) x (100 / C)) - % Clay

% Coarse Silt (0.05 – 0.02 mm) = 100 - % Sand - % Fine silt - % Clay

% Sand (2 – 0.05 mm) = E x 100 / C

Where, A is the weight of clay, B weight of sodium hexametaphosphate in the volume of the suspension taken, C and D are the weight of sample and fine silt respectively and E is the mass of sand fraction. The factor of 50 is derived from cylinder volume / in the volume of the suspension taken (500/10).

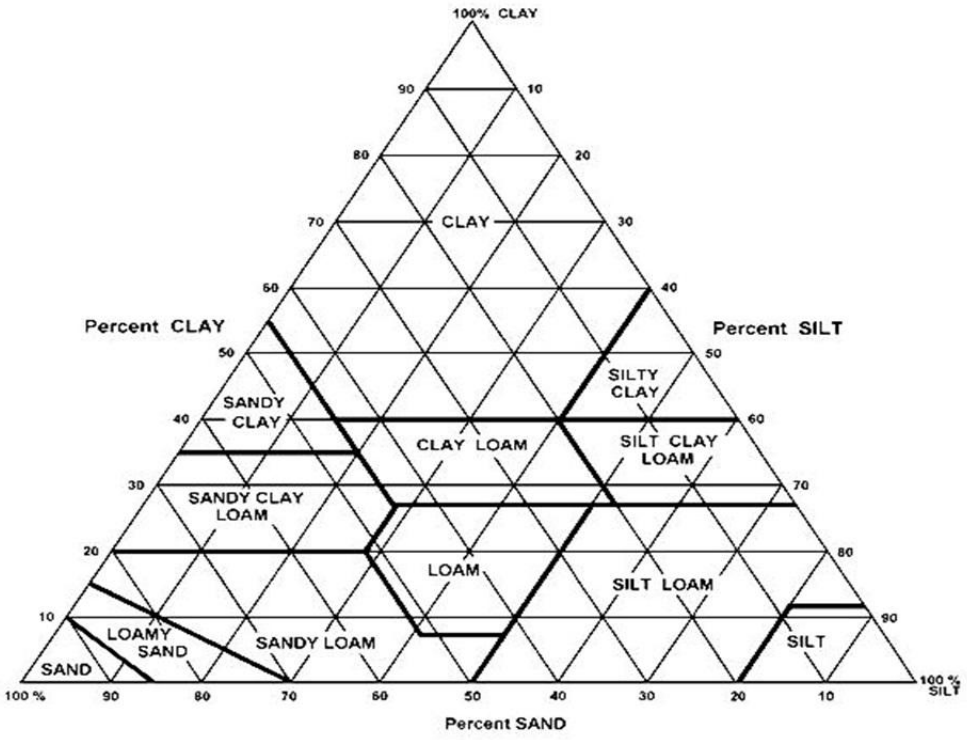


Fig. 7. The soil texture triangle.

2.4.1.2. Soil bulk density (D_b g cm^{-3})

The D_b is a dynamic soil property and is strongly influenced by the quantity and size of the pore spaces as well as the composition of the solid soil materials. Consequently, loose, porous soils will have lower bulk densities than more compact soils. Soil bulk density was estimated by the core method (Hao et al., 2008). Clean, dry, and uniform cylinder with a known volume (45 cm^3) was applied in order to remove the undisturbed soil core. The content of the cylinders was pushed out into a preweighed porcelain evaporating dish and soil samples then placed in an oven set to 105 ˚C for 48 h. The weight of dry soil samples plus porcelain evaporating dish was recorded, after drying and cooling in a hosphate n, and BD was calculated through the following equation.

Soil bulk density (g cm^{-3}) = Oven-dried weight of soil / Volume of soil

2.4.1.3. Soil porosity (%)

Soil porosity is influenced by texture, structure (e.g. degree of aggregation), and organic-matter content. For instance, coarse-textured soils have larger pores than fine-grained soils, which allow for more water flow. Organic matter greatly increases the water-holding capacity of a soil (Haney and Haney, 2010). Soil porosity was calculated with the formula given below.

Soil porosity (%) = Soil bulk density / 2.65

2.4.1.4. Water-Filled Pore Space (WFPS %)

WFPS was obtained using the soil water content, bulk density, soil volume, volumetric water content, and soil porosity. The following equations were used in our calculations (Haney and Haney, 2010):

Soil water content (g/g) = Weight of moist soil – Weight of oven-dried soil/ Weight of oven-dried soil

Volumetric water content (g cm^{-3}) = Soil water content x bulk density

WFPS (%) = volumetric water content x 100/ Soil porosity

2.4.1.5. Available water capacity (m/m)

The available water capacity is an indicator of a soil's water storage capacity in the field, which is important for plant growth. Clay soils tend to keep more water than sandy soils, they naturally have high water retention ability, and therefore in heavier soils the available water capacity is less critical (Appendix 1).

AWC was calculated according to the following equation:

AWC=1.475 – 0.010 (% S) + 0.011 (% L) + 0.138 (% C)

Where, S, L and C are amount of Sand (%), silt (%) and organic carbon (%) respectively.

2.4.1.6. Aggregate size stability distribution

Aggregate size stability was determined using the proposed methodology by Ma´rquez et al. (2004). In this method aggregate-size fractions were isolated by wet sieving using air-dried 8-mm sieved soil. Two 80-g subsamples of air-dried soil were used to analyze the aggregate-size stability distribution. Two pretreatments were applied before wet sieving: air drying followed by rapid immersion in water (slaked) and air drying plus capillary rewetting to field capacity plus 5% (capillary-wetted) (Six et al., 1998).

It is believed that aggregate stability is maximum at a moisture content of field capacity plus 5% (kg / kg). Slaking disrupts aggregates due to internal air pressure and aggregates that resist slaking are more stable than rewetted aggregates (Gale et al., 2000; Six at al., 1998; Cambardella and Elliote, 1993).

Aggregates were physically separated in four aggregate-size fractions:

- Large macroaggregates >2000 µm in diameter
- Small macroaggregates between 2000 and 250µm in diameter,
- Microaggregates between 250 and 53µm in diameter
- The mineral fraction <53 µm in diameter.

After wet sieving, all the fractions were oven-dried at 70 ˚C, except the large and small macroaggregates obtained by the capillary-wetted pretreatment. These macroaggregates were air dried and later used for the separation of large and small stable macroaggregates (subsequent slaking step) (Fig. 9). Sand corrections were performed by subtracting the total sand content of each size fraction from the amount of sample retained on each size fraction.

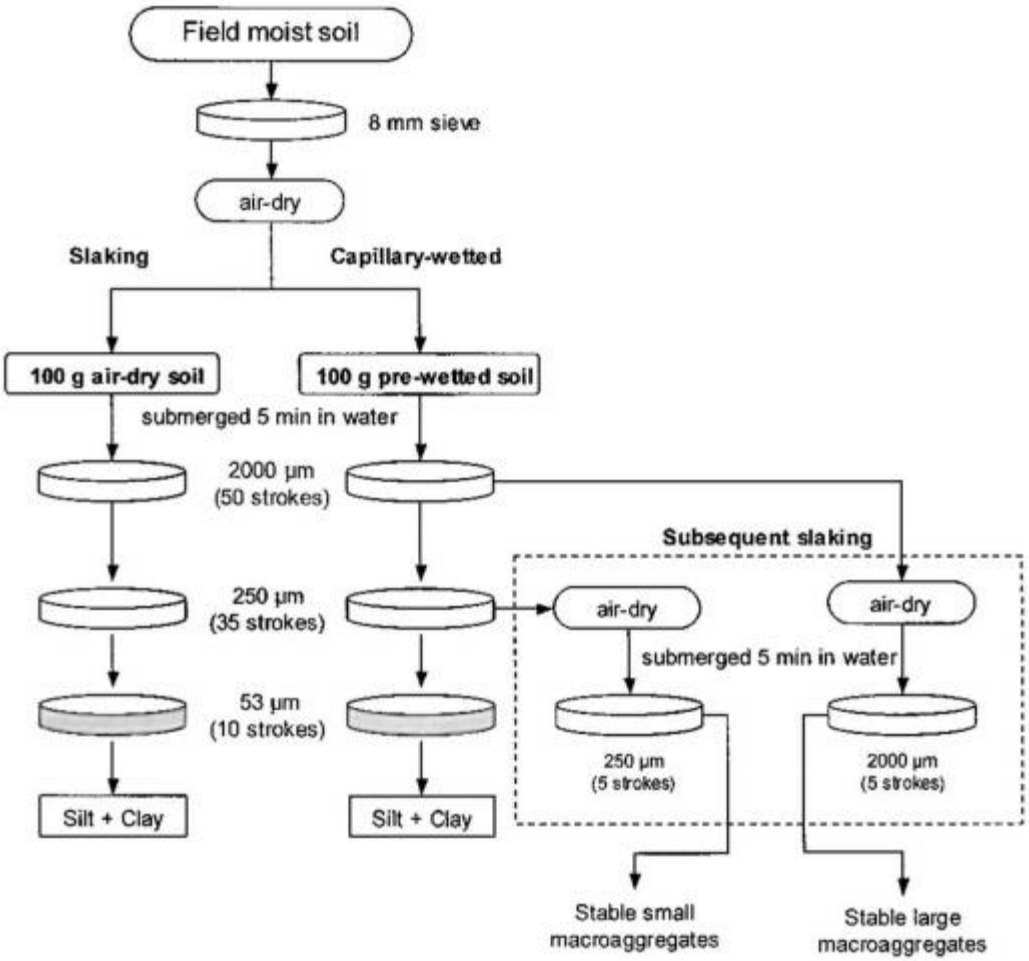


Fig. 8. Experimental procedure used to assess aggregate-size stability distribution. Source: Ma´rquez et al. (2004).

The total sand content of each aggregate-size fraction was determined by weighing the material that was retained on the sieve with a 53 µm screen upon dispersal of the aggregates with sodium hexametaphosphate (5 g L^{-1}), 5 grams sub-sample from each size fraction obtained were stirred for 18 h with 40 ml of sodium hexametaphosphate through reciprocating shaker. Appendix 2 shows the indices that have been used for assessing soil stability.

2.4.2. Chemical indicators

2.4.2.1. Soil pH in water

Soil pH is one of the most important measurements in standard soil analyses. Many soil

Chemical and biological reactions are affected by the pH of the soil solution. Soil pH was analyzed in (1: 2.5) soil: water suspension using glass electrode (Violante and Adamo, 2000).

2.4.2.2. Soil organic carbon

The soil organic carbon (SOC) contents of the soils were determined by the potassium dichromate oxidation-volumetric method according to Walkley and Black (1934).

$$2H_2Cr_2O_7 + 6H_2SO_4 + 3C \rightarrow 2Cr_2(SO_4)_3 + 3CO_2 + 8H_2O$$

Total organic carbon g kg^{-1} = 3.9× (blank titration – sample titration)/ weight of sample × Iron molarity

Organic matter g kg^{-1} = total organic carbon g kg^{-1} × 1.724

2.4.2.3. Cation exchange capacity

Cation exchange capacity (CEC) has a major effect on soil chemical, physical and biological properties, and it is used as a measure of fertility, nutrient retention capacity, and the capacity to protect groundwater from cation contamination. It has long been known that the cation exchange capacity varies depending on soil properties (e.g. pH, O.M, and texture). Clay and organic matter due to their electrostatic surface charges and large specific surface area have an important role in soil CEC (Havlin et al., 2009). This holding capacity varies for the different clay types (meq/100 g: vermiculite: 100 to 500; smectite: 70 to 95; illite: 10 to 40; kaolinite: 3 to 15) and clay-blends present in soil, and is very dependent on the proportion of clay and organic matter (meq/100 g: humus: 200) that is present in a particular soil. According to Mirkhani et al. (2005) cation exchange capacity of soils ranges from less than a centi-mol per kg ($cmol^+$ kg^{-1}) in sandy soils with low organic matter to more than 25 $cmol^+$ kg^{-1} in clay soils with high organic matter.

The CEC of soil samples was determined by EDTA titration after treatment with barium chloride and triethanolamine (Gessa and Ciavatta, 2000). The following equation was used to determine the CEC values ($cmol^{+}\ kg^{-1}$) according to the amount of magnesium adsorbed, which corresponds to the amount of barium exchanged:

$$CEC = (V_B - V_T) \times 0.25 \times (25 + B - A) / M \times 2$$

Where V_B is expressed as a volume of EDTA solution (ml) used for titration of the blank solution, V_T is volume of EDTA solution (ml) used for titration of the sample solution, A is the mass of the centrifuge tube plus sample (g), B is the mass of centrifuge tube plus the sample after saturating with barium chloride and washing with distilled water, M is the mass of the sample (g).

2.4.2.4. Electrical conductivity in water (1: 5)

Electrical conductivity ($dS\ m^{-1}$) was measured in water (1: 5) using conductimeter. 20 g of 2-mm-sieved soil sample was shaken mechanically for 2 h in 100 ml distilled water to dissolve soluble salts, after settling the suspension for at least 30 min or long enough for the solids to settle, filtered through Whatman No.42. The following equations were applied to calculate the level of soluble salts:

$$Ls = G.\ F.\ k$$

Where Ls is the electrical conductivity of the sample at 25 $^{\circ}C$, G is measured EC of suspension, F is the temperature correction factor, K is dilution ratio.

2.4.2.5. Total Carbonates

A variety of methods can be used for the determination of calcite in soils. In the current study total carbonates were determined by gas-volumetric method using a calcimeter (Boero,

2000). In this method a known quantity of 37% Hydrochloric acid in solution diluted 1:1 (volume / volume) is consumed by reaction with the preweighed soil sample carbonates (Fig. 10). The volume of CO2 released, by treating acid, is used to calculate the calcium carbonate content of soil (g kg^{-1}).

2.4.2.6. Extractable phosphorus (P)

Extractable phosphorus (P) was determined by extracting samples with a 0.5 N sodium bicarbonate solution, reacting the extracts with NH4-molybdate and determining P concentrations with a spectrophotometer (Olsen et al, 1954).

In this method 5g soil was shaken mechanically for 30 min in 100 ml extraction reagent (0.5 M $NaHCO_3$) at pH 8.5 and immediately filtered through Whatman n.42 then collected 5 ml of the extract for P concentration determination. This was followed by adding 0.5 ml sulfuric acid 2.5 M to the extract which was transferred into the centrifuge tube 50 ml, shaked until foaming stopped. Afterwards adding 8 ml mixed reagent (2.5 M sulfuric acid 50 ml + 0.1 M ascorbic acid solution 30 ml + 15 ml ammonium molybdate solution + 5ml antimony potassium tartrate solution), filling to 50 ml mark with distilled water. It let stand for 10 min to allow color to develop. Thereafter the colorimetric determination of the solution was made by spectrometer at λ = 880 nm. The calibration curve was made by standard solution of potassium dihydrogen phosphate (KH_2PO_4) (Fig. 11). Available phosphorus was obtained according to the following equation: P ppm = (P concentration in 50-ml sample flask) x (volume of the sodium bicarbonate extractant/ volum of soil extract used for P concentration determination)/ oven-dried weight of soil sample.

Fig. 9. Measuring total carbonate using calcimeter

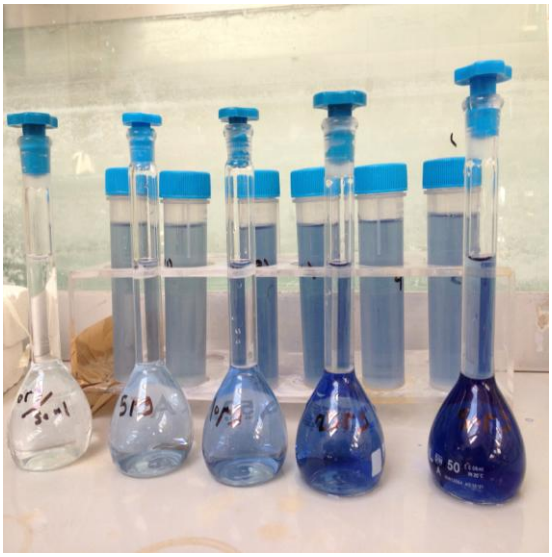


Fig. 10. P standard solutions to construct the calibration curve

2.4.2.7. Total element concentrations in Soil (ppm)

For analysis of total element contents including macro, micro and toxic elements in soil, air dried soil samples previously sieved to 2 mm were finely milled to 100 µm with an agate mill; 250 mg aliquot from each homogenized and powdered sample was mineralized with Aqua Regia (6 mL HCl 37% + 2mL HNO3 65% Suprapur, E. Merck, Germany, respectively) in a microwave oven (model Milestone 1200) in a Teflon vessel with specific soil digestion program according to Vittori Antisari et al. (2013). After cooling, solutions were made up to 20 mL with milli-Q water and then filtered with Whatman 42.

2.4.2.8. Total element concentrations in Plant (ppm)

For analysis of metals in rice root, straw, leaf and grains, according to the procedure recommended by Unterbrunner et al. (2007) and Fontana et al. (2010), 0.5 gr of milled sample was digested in an acid mixture of 5 mL 65% HNO_3 and 3 ml 30% H_2O_2 in open vessels on the hot plate, followed by filtration with filter cellulose Wathman n. 42 as explained by Zang et al.

(2002) and Jones (2001) whit slight modifications. For the instrumental method accuracy and the analytical results quality the soil and rice samples were prepared in duplicate and the International Reference Materials (BCR-CRM 141R, 142R, 143R for the soils; BCR-CRM 060 and 062 for the plants) provided by the European Commission were used. Contents of 22 elements (Al, As, Ba, Be (not determined for plant), Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, extractable P, Pb, Sb, Si, Sn, Sr, Ti, TI, V and Zn) were determined by Inductive Coupled Plasma Optical Emission Spectrometry (ICP-OES, Spectro Ametek, Arcos) for both plant and soil samples.

2.4.3. Biological, Biochemical and microbiological indicators

2.4.3.1. Potentially mineralizable Nitrogen

Potentially mineralizable nitrogen is considered as an important indicator of the soil quality, as it reflectss the capacity of the soil microbial community to provide available nitrogen to the plants. Estimate of the N mineralized from SOM will improve the sustainability of agriculture, since it allows farmers to determine the rate of N fertilizer application required to optimize crop yield and to minimize N losses to the environment (Ross et al., 2011). Potentially mineralizable nitrogen (µg gr $^{-1}$ week^{-1}) was determined based on Cornell Soil Health Assessment Training Manual (Guginoet al., 2007). According to this method after soil sampling, the mixed composite soil samples were refrigerated (about – 10 $^{\circ}$C), after two weeks sieved and two soil samples (8g) were taken and put into 50 ml centrifuge tubes. 40 ml of 2 M potassium chloride (KCl) was added to one of the tubes, shaken on mechanical shaker for 1 hour, centrifuged for 10 minutes. Thereafter ammonium concentration was analyzed applying 20 ml of the supernatant (time 0 mesurment) using spectrophotometer at λ = 640 nm; A standard curve was established using ammonium chloride NH_4Cl standard solutions. 10 ml of distilled water was added to the second tube, it was shaken manually, incubated for 7 days at 30°C. After the 7

day anaerobic incubation 30 ml of 2.67 M KCl was added to the tube, shaken on a mechanical shaker for 1 hour, centrifuged for 10 minutes and then 20 ml of the supernatant was used to analyze ammonium concentration after 7 day. The difference between the time 0 and time 7-day ammonium concentration was considered as the rate (microgram nitrogen mineralized per gram dry weight of soil per week) at which the soil communities are able to provide inorganic nitrogen into plants.

2.4.3.2. Active Carbon

Active carbon is the liable fraction of soil C that is readily available as a carbon and energy source for the soil microbial community. Fractions of SOC that are thought to represent the active C pool, and serve as sensitive indicators of changes in management-induced soil quality, include microbial biomass carbon, rapidly mineralizable carbon, particulate organic matter, and extractable soil carbohydrates (Gugino et al., 2007; Weil et al., 2003).

Active carbon was determined following the methodology proposed by Weil et al. (2003). From the mixed composite soil samples which were stored at -10 °C, a subsample was taken to air dry. The soil was ground and sieved to 0.5 mm. A 2.5 g sample of sieved soil sample was placed in a 50 ml centrifuge tube filled with 20 ml of a 0.02 M potassium permanganate solution, which is deep purple in color. Loginow et al. (1987) stated that in a natural to slightly alkaline solution, $KmnO_4$ is a powerful oxidizing agent; at pH 7.2 portion of SOC react with $KmnO_4$ to partially bleach the deep purple permanganate color to light pink or clear. The soil and $KmnO_4$ were shaken for 2 min through mechanical shaker in order to oxidize the “active” carbon in the sample (Fig. 12).

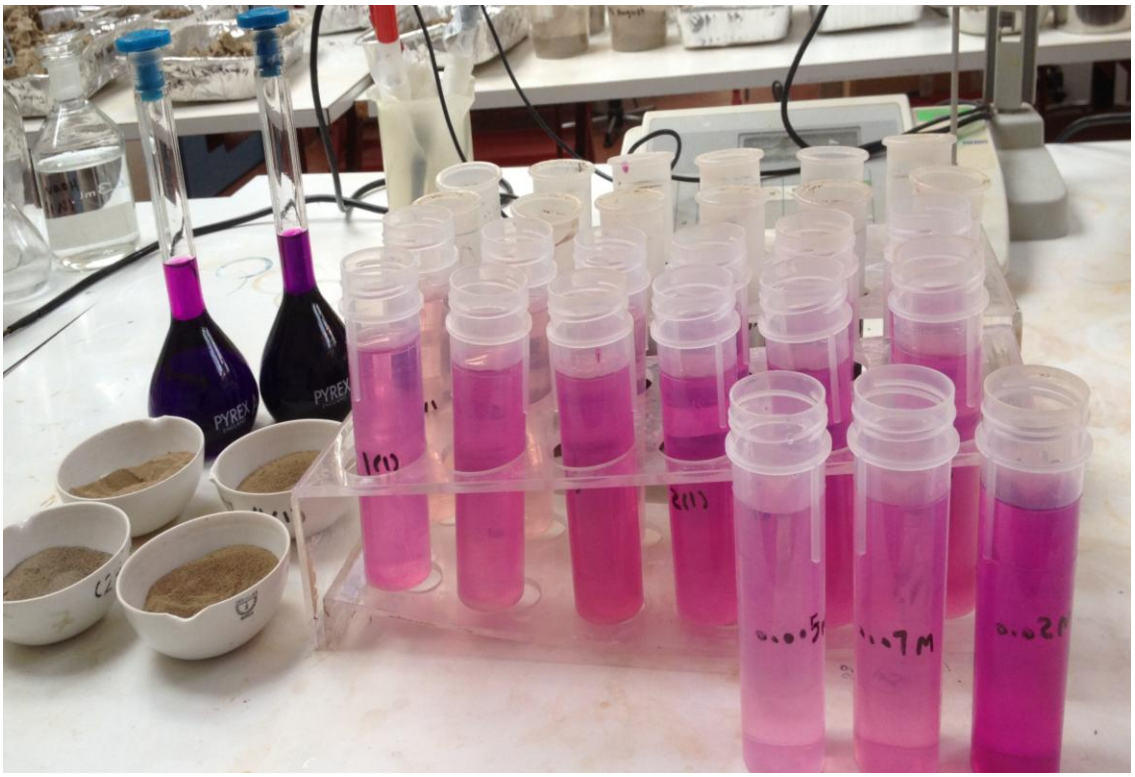


Fig. 11. Oxidation of the active carbon in the studied soil samples by 0.02 M potassium permanganate solution; the purple color became lighter as a result of this oxidation

The sample was centrifuged for 5 min, and the supernatant was diluted with distilled water and absorbance measured at 550 nm. A standard curve was constructed using 0.5 ml of the 0.005, 0.01, and 0.02 $KmnO_4$ solutions plus 45 ml distilled water. The following equation was used to convert sample absorbance value to active C in units of mg C per kg of soil.

$$\text{Active C (mg kg}^{-1}) = [0.02 \text{ mol/l} - (a + b) \times \text{absorbance})] \times (9000 \text{ mg C/ mol}) \times (0.02 \text{ l solution} / 0.005 \text{ kg soil})$$

Where 0.02 mol/l is the initial solution concentration, a is the intercept and b is the slope of the standard curve, 9000 is mg C (0.75 mol) oxidized by 1 mol of MnO_4 changing from Mn^{7+} to Mn^{4+}, 0.02 l is the volume of $KmnO_4$ solution reacted, and 0.005 is the kg of soil used.

2.4.3.3. Soil respiration

Soil respiration (SR) or carbon mineralization, is referred to carbon dioxide release from the soil surface. It is the biggest carbon efflux process after photosynthesis in the terrestrial ecosystem (Fang and Wang, 2007). Soil respiration was measured using the titration method. A 20 g wet soil sample, within a range of 50-60 % WFPS, and 4ml of 1N NaOH were placed separately into plastic containers within 500 ml glass jar for an incubation period of 28 days at about 25 °C. The amount of carbon dioxide trapped in NaOH was determined in 1, 4, 7, 21, and 28 days, adding 8 ml of 10 % $BaCl_2$, 5 drops of Phenolphthalein indicator and titrating the solution with 0.1 N HCl. The endpoint of the titration was considered when the color changed from pink to white. The content of mineralized carbon expressed as released CO_2 was calculated using the following equation (MIPAF, 2000):

$$mg\ C - CO_2\ g_{s.s.}^{-1}\ h^{-1} = \frac{(V_o - V) \times M \times E}{20 \times h_{inc}}$$

Where V_o and V are the volume of HCl used to titrate the blank and the sample, M is the molarity of HCl, E= 6 is the equivalent weight of carbon in CO_2 and h is the incubation time (hours).

2.4.3.4. Soil enzymatic assays

Indeed, soil enzymes have the potential to reflect the effects of environmental changes caused by natural or anthropogenic origin (e.g. land waterlogging, different crop rotation, land use changes, application of pesticides, herbicides, organic and inorganic fertilizers, tillage) on soil quality. Moreover, their activity measurement is rapid, easy and inexpensive (Aon et al., 2001; Raiesi and Beheshti, 2014).

Six soil enzymatic activities involved in C, N, P, S cycles were determined (β-glucosidase, leucine-amminopeptidase, chitinase, alkaline and acid hosphate n and arylsulfatase) as

described by Cowie et al. (2013). Briefly, enzymes were desorbed through heteromolecular exchange using 0.5 g of air-dried soil.

2.4.3.5. DNA measurement

The dsDNA as a measure of soil microbial biomass was carried out according to Ventura et al. (2014). A 0.5 g of well mixed, 0.5 mm sieved, and air dried soil samples were placed in 2ml tubes together with glass microbeads and 1 ml of sodium phosphate solution. The tubes were then agitated for 3 minutes through the mill (Model: Retsch MM400) at a frequency of 30 strokes per second and subsequently centrifuged at 20 000 g for the duration of 3 minutes. The content of dsDNA was determined in the supernatant by fluorimetry on microplate using the specific fluorophore PicoGreen reagent (Life Technologies), which binds the DNA double helix selectively. The assay was performed following instructions given by the producer house, and results were recorded by a microplate lecturer (Synergy HT, Bio-Tek; software Gen 5). Determination was repeated twice.

2.4.3.6. Biological quality of soil index: micro-arthropod method (QBS-ar)

New methods, based on soil microarthropods for soil quality evaluation have been proposed by some Authors. Soil microarthropods demonstrated to respond sensitively to land management practices and to be correlated with beneficial soil functions. Parisi et al., 2004 proposed a soil quality index based on soil microarthropods. The QBS Index (QBS-ar) is calculated on the basis of microarthropod groups present in a soil sample. Each biological form found in the sample receives a score from 1 to 20 (eco-morphological index, EMI), according to its adaptation to soil environment (Appendix 3).

The QBS-ar scores can be transformed into 7 soil quality classes, as given in Appendix 4. The increasing values of the classes correspond to more complex and soil-adapted

microarthropods communities. Protura, Onychiurid Collembola and Coleoptera are three taxa that play a major role for the transformation processes (Parisi et al., 2004).

After collecting the surface soil samples (0 – 15 cm depth) from different rotation systems, they were carried into the lab protected by thermal shock and microarthropods were extracted within 48 hours from sampling by a Berlese-Tullgren funnel (25 cm diameter, 2 mm mesh, 60 W lamps at ca. 25 cm distance) over a period of 15 days. The soil core was delicately placed on the mesh above the funnel and all of the soil that fell during sample disposal was put onto again on the mesh before inserting a bottle of preservative liquid (ethanol 75%) beneath the funnel. The extraction system was kept away from vibrations and other disturbs (Fig. 12 and 13). Extracted specimens were observed under a stereomicroscope at low magnification (20-40 x) in the same preservative liquid, pouring the animals and the liquid in Petri dishes. The QBS score of the samples were calculated by summing up the EMIs of all groups collected from each rotation system.

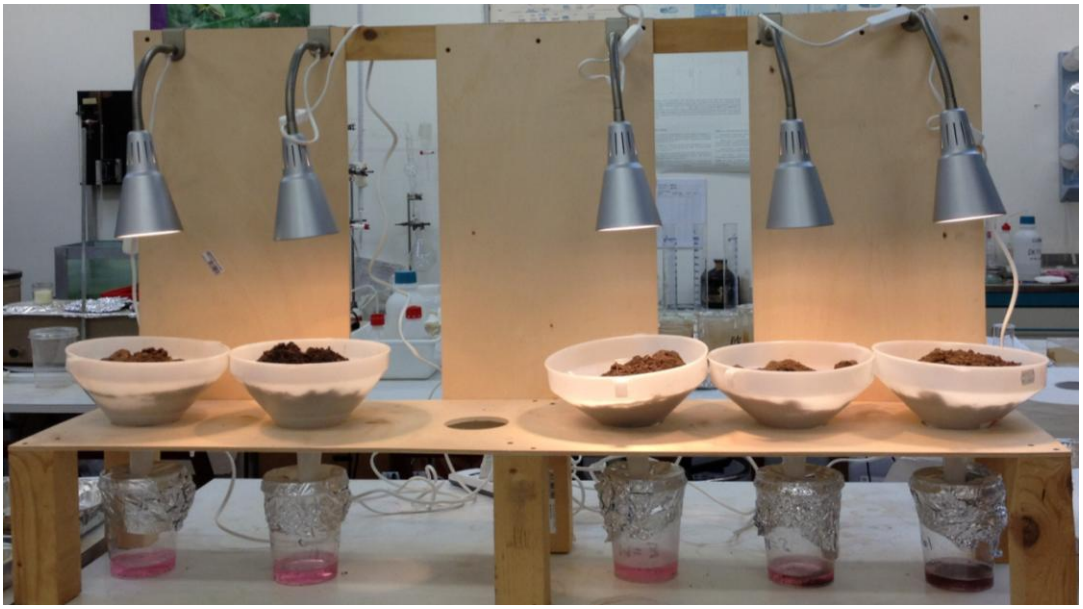


Fig. 12. The apparatus for the extraction of microarthropods in order to determine the QBS-ar index (Venice, Italy)

Fig. 13. The apparatus for the extraction of microarthropods for the determination of the QBS-ar index (IRRI, Manila)

2.5. Assessment of soil quality

In this research work soil quality assessment was accomplished using three different soil quality indices, namely, an additive index based on soil management assessment framework, a weighted additive index and systematic soil quality index. Each soil quality index was calculated using a minimum data set (MDS) chosen based on principal component analysis (PCA) (except of systematic soil quality index) and expert opinion using two scoring methods (non-linear scoring method against linear scoring method). In other words, soil quality indices were computed based on three main steps: indicator selection, interpretation of indicator, and

integration indicator scores into a SQ index. These steps are described in detail by the following subsections.

2.5. 1. Indicator selection

To select a minimum data set from large data sets, two main methods, namely, expert opinion (EO) and statistical multivariate data reduction technique standardized principal component analysis (PCA) were used in this research.

2.5.1.1. Indicator selection based on EO

Expert opinion, by definition, needs expert knowledge of the system and selecting MDS has relied primarily on EO; therefore in this method the minimum data set should be chosen according to the consensus of the other research works, recommendation in the literature and of course common management concerns in the study area (Karlen et al, 2008; Andrews et al., 2002a; Doran and Parkin, 1994; Larson and Pierce, 1991).

Andrews et al. (2002a) showed that both expert opinion and multivariate statistic selected MDS indicators describe equally the variation of management goals (i.e. endpoints).

In the SMAF, the indicator selection step applies an expert system of decision rules to suggest indicators for entry in the assessment. According to the management goals, location and current practice, the program identifies the functions as important to that goal. Subsequent, a list of indicators is selected; those are associated with each identified soil function. Finally, the indicators are further narrowed by applying various additional criteria such as: crop or rotation, organic matter class, texture, slope etc.

2.5.1.2. Indicator selection based on PCA

There are various technics for using PCA to choose a subset from an initial large data set; the one used in this research work is similar to that defined by Dunteman (1989) and Andrews

and Carrol (2001). Principal components (PCs) for a data set are described as "linear combinations of the variables that account for maximum variance within the set by describing vectors of closest fit to the n observations in p-dimensional space, subject to being orthogonal to each other" (Dunteman, 1989). As described in Andrews et al. (2002a) it was assumed that PC_S obtaining high eigenvalues and variables with high factor loadings best represent variation in the systems. The PC_S with eigenvalues ≥ 1 were thus assayed. Within a particular PC, each variable is given a factor loading that indicates the contribution of that variable to the composition of the PC. Under each principal component, only the highly weighted factors, those with absolute values within 10% of highest weight, were kept for the MDS. When more than one factor was maintained under a single PC, they were then subjected to multivariate correlation coefficients to reduce the redundant variables by eliminating them from the MDS (Andrews et al., 2002 a). If the highly weighted factors were not correlated (assumed to be a correlation coefficient < 0.60), then each was considered important, and thus, retained in the MDS. In order to choose variables among well-correlated groups (> 0.70), the absolute values of the correlation coefficients were summed for these variables. It was assumed that the variable having the highest correlation sum best represented the group. Andrews et al. (2002 a) mentioned that the choice among well-correlated variables could also be based on practicality (i.e., ease of sampling, cost, and interpretability).

2.5.2. Indicator transformation

This step is the most complex and most important in soil quality evaluation. After selecting appropriate indicators for MDSs either by Expert opinion (EO-MDS) or Principal component analysis (PCA-MDS), indicators are interpreted using scoring curves. The indicator interpretation step involves transformation of each observed MDS indicator values into the

unitless values (0-1) that demonstrate the associated level of function in that system. An indicator score of 1 represents the highest potential function for that system, that is, the indicator is non-limiting to pertinent soil functions and processes, within the soil's inherent capability (Andrews et al., 2004). Schiller et al. (2001) stated that the use of scoring curves for data analysis and synthesis allows interpretations to reflect both ecosystem function and farmer and societal values regarding crop production and environmental protection.

In general, the interpretation of indicators is accomplished based on three types of standardized scoring function: (1) 'More is better', (2) 'Less is better' and (3) 'Optimum'. The shape of an indicators scoring curve is determined by the relationship between the indicator and the soil functions. 'More is better' curves score those properties that are related with improved soil quality at higher levels (e.g. aggregate size stability, active carbon, soil respiration, cation exchange capacity). 'Less is better' curves score soil quality indicators that indicate poor soil quality at high levels (e.g. bulk density). 'Optimum' curves score soil properties that have an increasingly positive influence on soil quality up to an optimal level beyond which their influence is detrimental (e.g. porosity, water-filled pore-space, extractable phosphorous, pH, electrical conductivity) (Karlen et al., 1994, Glover et al., 2000, Andrews and Carrol, 2001; Andrews et al., 2002 b, Andrews et al ., 2004). Two techniques can be used for scoring MDSs: linear scoring and non linear scoring (Andrews and Carroll, 2001; Andrews et al., 2002a and b; Andrews et al., 2004).

2.5.2.1. Linear transformation of indicators

Linear scoring curves for 'More is better' and 'Less is better' were generated from the following equations respectively (Andrews et al., 2002a).

LSF (Y) = x / h

LSF (Y) = l / x

Where, Y is the linear score, x the soil property value, h the highest observation value such that the highest observed value receives a score 1, and l the lowest observed value such that the lowest measured value takes a score of 1. A combination of both equations was calculated for "Optimum" scoring function, that is, these indicators (e.g. phosphorus, water-filled pore space, porosity**)** are scored as "more is better" up to a threshold amount then scored as "less is better" beyond threshold. In this research, critical values (thresholds) for each soil quality indicator are based on the critical values which has been proposed in the literature (expert opinion), or measured values observed under near-ideal soil conditions for the study area.

2.5.2.2. Non-linear transformation of indicators

Selected minimum data sets were transformed into non-linear scores using the soil management assessment framework. According to the soil management assessment framework, each indicator-scoring curve is an algorithm or logic statement (e.g., if, then, else) made up of parameters and coefficients that describe it (Wienhold et al., 2009). "The parameters for each algorithm do not change on the basis of site-specific factors, such as soil type, crop, or climate, and are termed "fixed parameters". In contrast, in the scoring curve algorithms that change to best represent the relationship between indicator and soil function (s) under differing conditions or systems are called site-specific parameters. The expected range for each indicator is determined using site specific factors. In other words, the range for the indicator varies according to the site-specific factors. The algorithms are quantitative relationships between empirical values of measured indicators and normalized scores, reflecting the performance of ecosystem service (s) or soil function" (Andrews et al., 2004).

In our research the shapes of most indicator curves are specified based on literature review and consensus of collaborating researchers. The SMAF was applied for Bulk Density (BD), Water-filled pore space (WFPS), β-glucosidase (BG), Potentially Mineralizable Nitrogen, Soil Organic Carbon (SOC), Stable Aggregate Index (SAI), pH, electrical conductivity. However, the current version of the SMAF has scoring curves for 13 soil properties but more than 60 other properties have been identified as having potential as assessment indicators (Wienhold et al., 2009).

The soil indicators for which a scoring curve was not developed, in the current version of SMAF, a general linear scoring curve (NLSC), as stated in Wymore (1993) and Karlen and Stott (1994) was used.

The scoring curves were therefore generated for the rest of indicators, the MDSs which were not included in the SMAF, such as Porosity, Active Carbon, Soil respiration, QBS-ar, Total Nitrogen, Extractable P based on the following equation:

$$NLSC: score(y) = \frac{1}{\left[1 + \left(\frac{B - LT}{x - LT}\right)^{2S \times (B + x - 2LT)}\right]}$$

Where B is the baseline value of the soil indicator where the score equals 0.5, L is the lower threshold, S is the slope of the tangent to the curve at the baseline, x is the soil indicator analytical value. The thresholds are taken from literature.

Where we could not find a well suited set of thresholds, concerning the paddy fields system, the scoring curves were generated, using the data set obtained from analytical procedure, namely, measured data were graphed versus normalized observed data using CurveExpert fitting software, version 1.4 to obtain the best model. CurveExpert compares the fit of data to a

library of available models, selects the model having the lowest root mean square error and provides coefficient estimates for the model giving the best fit (Wienhold et al., 2009).

It is usually assumed that the general shape of the relationship between indicator and a soil function holds among agroecosystems, while the range for each indicator often varies across systems. It is believed that the variation from system-to-system results from differences in site-specific factor such as climate or inherent soil properties (Wienhold et al., 2009).

2.5.3 Indicator integration into indices

This step depends on the scored indicators, which were obtained from indicator transformation step either by linear or non-linear transformation, since the indicator measurements must be transformed into unitless values before they can be meaningfully combined. While the first two steps are the most critical, step 3 allows one to see the overall health of the soil, without distraction of (potentially) conflicting individual indicator results (Andrews et al., 2004).

Three soil quality indices were used in this study: an additive SOI (ADD SQI) (Andrews et al., 2002a, 2004; Andrews and Carroll, 2001; Masto et al., 2008; Schindelbeck et al., 2008, Idowu et al., 2009) ; a weighted additive SQI (WTD SQI), and systematic soil quality index for soil ecological functions, the one proposed by Karlen and Stott (1994) and used in many soil health studies (Glover et al.,2002; Andrews et al., 2002a; Lima et al., 2008; Lima, 2007). However, Andrews et al. (2002a) found few differences among various integration techniques including additive, weighted; and max–min objective functions (e.g. Yakowitz et al., 1993) when used to combine nonlinearly scored indicator values.

2.5.3.1. Additive Index

Once the scored indicators, from the two MDSs (EO or PCA), were obtained, this step is accomplished by summing the scores for each indicator, dividing by the total number of indicators.

$$ADD - SHI = \frac{\sum_{i=1}^{n} S_i}{n}$$

Where S_i represents the scored indicator value obtained either by the linear or no-linear transformation of MDS indicators and n is the total number of indicators in the MDS.

Andrews et al. (2003) suggested using the number of indicators in the MDS as a divisor corrects for any missing data in the data set.

2.5.3.2. Weighted additive index

As stated in Andrews et al. (2002a), after transforming, the MDS variables for each observation were weighted using the PCA results. Each PC illustrated a certain amount (%) of the variation in the total data set. This percentage, divided by the total percentage of variation explained by all PCs with eigenvalue > 1, provided the weighting factor for variables selected under a given PC. We therefore summed the weighted MDS variable scores for each observation in the following formula:

$$\mathrm{SQI} = \sum_{\mathrm{i}=1}^{\mathrm{n}} W_{\mathrm{i}} \times S_{\mathrm{i}}$$

Where W is the weighting factor derived from the PCA and S is the indicator score. Andrews et al. (2002a) believed that weighting the EO MDS using PCA weights is slightly artificial, since one of the advantages of the EO method is that preliminary statistics are unnecessary. Nevertheless, as Andrews et al. (2002a), we used the weighting factors derived

from the PCA for EO MDS as well. Consequently using the same weights among SQIs allowed us for better comparisons.

2.5.3.3. Systematic soil quality index

Another weighted additive soil quality index, the one was proposed by Karlen et al. (1994) was also considered in this research. In this approach soil quality index was therefore calculated using six ecological soil functions, which were weighted and integrated according to the following expression:

$$WTD_{SF} - SHI = q_{ps}\,(wt) + q_{wr}\,(wt) + q_{nc}\,(wt) + q_{fb}\,(wt) + q_{bh}\,(wt)$$

Where Wt is the numerical weight assigned for each soil function according to the soil function's importance in fulfilling the overall goals of maintaining soil quality under specific soil condition and land-use propose (Glover et al., 2000). Q_{ps} is the rating for the soil's ability to resist degradation (soil physical stability and structural support), q_{wr} is the rating for the soil's ability to regulate water transfer, absorption, drainage and storage nutrients and compounds dissolved in the water (water relations), q_{nc} is the rating for the soil's ability to sustain plant growth (nutrient cycling), q_{fb} is the rating for the soil's ability to filter and buffer high concentration of nutrients and pollutants (Filtering and Buffering), q_{bh} is the rating for the soil's ability to provide habitat for soil biota functional groups (Biodiversity and Habitat).

Associated with each soil function there are soil health indicators which influence the related function. Level 1 indicators are most directly associated with the soil function, whereas indicators of level 2 are associated with several subfunctions. Assigned numerical weights must sum up to 1.0 at each level.

The score for a specific soil function obtained through multiplying each indicator score (obtained from the linear and non-linear transformation) by its weight and summing up these

values. Eventually, each soil function score was multiplied by its weight and these values were then summed to give an overall assessment of soil health, reflecting management practice effects on the whole soil functionality. This weighted additive soil quality index was considered as an ecosystem performance index, where the soils managed in a certain way are able to supply this ecological function at maximum capacity, without constraints (Hussain *et al*., 1999).

2.5.4. Endpoints

Endpoints are outcomes driven by management or societal goals such as productivity, environmental protection, and waste recycling. The endpoint measures use as proxies for the identified management goals, applying to validate the efficacy of the selected MDS. For example if productivity is assumed to be the management goal for a case study, the endpoint measures might be selected as yield or net revenues (Andrews et al., 2002a and b; Andrews et al., 2004).

2.5.5. Statistical analysis

The following statistical analyses were performed in this research work:

- Descriptive statistics of observed soil quality indicators
- A two-way analysis of variance (ANOVA) on the measured physical, chemical and biological soil parameters in order to identify indicators with significant treatment difference, using the LSD test calculated at $P < 0.05$.
- Pearson correlation coefficients between most investigated soil quality indicator pairs in the study area
- The principal component analysis to establish a minimum soil quality dataset from 62 soil variables.

- Stepwise multiple linear regression analysis of observed and scored indicator values (as independent variables) against end-point variables (as dependent variables) to examine the ability of both the EO and the PCA selected-MDSs to explain variability in end-point data representing sustainable management goals.
- A two-way analysis of variance (ANOVA) on the soil quality index outcomes (Additive Index, Weighted additive index, systematic soil quality index) to compare soil quality indices, using the LSD test calculated at $P < 0.05$.
- The Pearson correlation coefficients of index outcomes, with particular soil indicators, and end –point variables, in order to understand the level of correspondence and the direction of alterations.

We used Statistica 7 to perform the analysis of principal component analysis (PCA). CurveExpert 1.4 was used to obtain the best fitting scoring algorithms, plotting the observed and scored values for each indicator, and also to model the scoring curve in response to site-specific factors. We used SPSS and SAS programs for other statistical analysis (i.e. a two-way analysis of variance (ANOVA), Pearson linear correlations, multiple linear regressions).

Chapter three

Results and discussion

"If we take care of the land, it will take care of us." ~Hugh Hammond Bennet

3.1. Effect of different cropping patterns on soil physical properties

Descriptive statistics of measured physical parameters, including Soil particle size distribution, Soil bulk density (BD g cm^{-3}), Soil porosity (%), Water-Filled Pore Space (WFPS %), Available water capacity (AWC m/m), and volumetric water content (VWC g cm^{-3}) of 48 soil samples are shown in Table 1. Soil bulk density is deemed as an important physical indicator of soil quality since it demonstrates the soil's ability to accommodate water and solute movement and soil aeration. In other words BD reflects concerns associated with soil compaction (Karlen et al., 2006; Glover et al., 2000); WFP, AWC, and VWC are physical indicators that could serve to evaluate soil function with regard to sustainable production since they are associated with plant growth and soil biological activity (Yao et al., 2013; Doran and Parkin., 1994). A wide range of values was found for most of the determined parameters; for instance, the soils had significant variability in particle size distribution (3.61 – 25.72 % clay, 18.96-62.18% silt, 15.87-70.86% sand), consistent with the parent material composition. Average Water-filled pore space of all soil samples was 0.64%, ranging from 0.18 to 1.09 %, with rather high coefficients of variation.

The results of two-way analysis of variance (ANOVA) of the above mentioned physical parameters of different rotation systems over the growing season are given in Table 2. Significant differences ($P < 0.05$) among different rotation systems at each sampling time were observed. Soil bulk densities were significantly lower at both April (after dry field preparation, field moist condition) and August (panicle formation, the late period of waterlogging) months in

F-R than in R-R-R, S-R-R and P-S-R rotation systems, which were not different from one another at either month. Nevertheless, R-R-R rotation had the highest degree of compaction in both April and October (BD= 1.45 and 1.58 g cm^{-3} respectively). However, a reverse trend was found for porosity (%), as the bulk density of a soil is inversely related to the soil porosity. The lower bulk densities measured in F-R rotation of this study are likely due to the high values of soil organic carbon in the months of April and August.

Table 1- Descriptive statistics of some basic physical soil properties

Rotation		**Soil physical properties**									
			Particle size distribution								
		BD (g cm^{-3})	Clay (%)	Coarse silt (%)	Fine silt (%)	Sand (%)	Texture	Porosity (%)	WFPS (%)	AWC (m/m)	VWC (g cm^{-3})
R-R-R	Mean	1.36	9.763	21.02	9.63	59.58	Sandy loam	51.03	0.52	0.14	0.15
	Max	1.65	13.01	28.12	15.92	63.47		62.11	1	0.15	0.24
	Min	1.04	6.69	11.92	4.10	55.80		38.01	0.27	0.13	.03
	CV (%)	15.08	20.75	21.72	33.17	3.98		16.39	47.18	5.22	0.47
											0.18
S-R-R	Mean	1.32	13.06	16.54	12.19	58.20	Sandy loam	49.50	0.58	0.15	0.36
	Max	1.48	15.99	23.38	20.71	68.27		55.67	0.99	0.18	.02
	Min	1	8.84	12.18	1.68	52.52		43.99	0.18	0.11	0.68
	CV	9.42	16.80	17.86	54.06	10.28		8.58	54.22	16.39	0.30
											0.61
F-R	Mean	1.23	4.81	18.38	17	59.81	Sandy loam	46.40	0.73	0.16	0.06
	Max	1.48	6.30	23.28	26.32	70.86		55.66	1	0.19	0.72
	Min	1	3.61	12.15	3.63	50.81		34.71	0.23	0.12	0.28
	CV	15.69	20.20	16.29	49.42	14.43		16.33	37.60	21.84	0.65
											0.22
P-S-R	Mean	1.34	21.48	26.63	29.46	22.43	Silt loam	51.31	0.76	0.21	0.50
	Max	1.45	25.72	47.11	42.93	25.45		54.25	1.09	0.23	0.15
	Min	1.12	19.11	15.21	15.07	15.87		46.24	0.43	0.20	0.24
	CV	6.86	9.04	37.47	33.21	11.09		3.94	27.13	4.23	0.03

Soil moisture regime is one of the most important factors for the control of soil properties. It contributes changing pH, Eh, organic matter degradation, enzyme dynamics and $CaCO_3$ content of soil (Van den Berg and Loch, 2000), and hence, may affect the transformation and repartition of heavy metals in soil, changing their availability to plants (Zheng and Zhang, 2011). The

volumetric water content (g cm^{-3}) in this study ranged from 0.03 in October to 0.68 in August, according to the flooding condition. Water-filled pore space in drained soil condition (October) was greater than those of the other months. The higher water-available capacity of P-S-R rotation as compared to other systems may have been due to the higher percentage of silt and clay particles (silt loam texture) in P-S-R rotation; it appears that the large surface area of the small soil particles allows the soil to maintain a higher amount of water.

Significant correlation ($P < 0.01$) was found between most investigated soil quality indicator pairs in the study area (Table 3). For instance, the highest positive correlations ($r > 0.50$) were observed for OC with DNA, and active carbon, and for DNA with PMN, and WFPS (see later, par. 3.5.1.). Strongest negative correlations ($r>0.50$) were observed for AC with, BD, and porosity and for AWC with sand.

Table 2- Multiple comparison of mean values of physical indicators (Soil particle size distribution (%), Soil bulk density (g cm^{-3}), Soil porosity (%), Water-Filled Pore Space (%), and Available water capacity (m/m)) among four different rotation systems over rice growing season. Data in a column followed by the same letter are not significantly different at $\alpha = 0.05$, two-way ANOVA with LSD test.

Cropping patterns	**Soil physical properties**							
April		**Texture**						
	BD (gcm^{-3})	Clay (%)	Coarse silt (%)	Fine silt (%)	Sand (%)	Porosity (%)	WFPS (%)	AWC (m/m)
R-R-R	1.45 a	11.83 b	18.03 b	8.64 c	61.48 a	54.80 a	0.42 b	0.15 c
S-R-R	1.36 a	14.51 b	15.20 b	11.29 c	58.99 a	51.52 a	0.39 b	0.13 c
F-R	1.10 b	5.40 c	19.42 b	24.33 a	50.81 b	41.86 b	0.77 a	0.18 b
P-S-R	1.35 a	21.06 a	40.96 a	17.39 b	20.58 c	52.79 a	0.49 ab	0.21 a
June								
R-R-R	1.07 c	11.06 c	19.97 a	8.95 bc	60 b	38.93 c	0.35 b	0.13 c
S-R-R	1.19 bc	13.85 b	16.84 a	16.08 b	53.21 c	45.09 b	0.25 c	0.15 b
F-R	1.39 a	5.44 d	19.03 a	7.34 c	68.17 a	52.55 a	0.30 bc	0.12 d
P-S-R	1.35 ab	20.15 a	17.43 a	39.83 a	22.57 d	52.28 a	0.71 a	0.21 a
August								
R-R-R	1.33 a	8.03 a	24.22 a	9.79 b	57.94 b	50.48 a	0.38 b	0.14 b
S-R-R	1.46 a	11.36 b	18.63 b	2.86 c	67.13 a	54.29 a	0.73 a	0.11 c
F-R	1.02 b	3.87d	18.69 b	24.97 a	52.45 c	37.75 b	0.93 a	0.19 a
P-S-R	1.28 a	24.29 a	27.48 a	24.31 a	23.90 d	49.71 a	0.88 a	0.19 a
October								
R-R-R	1.58 a	8.11 c	21.85 a	11.13 c	58.89 b	59.90 a	0.91 a	0.14 c
S-R-R	1.26 b	12.52 b	15.47 c	18.51 b	53.49 c	47.07 c	0.94 a	0.18 b
F-R	1.41 b	4.50 d	16.3 bc	11.34 c	67.79 a	53.42 b	0.92 a	0.12 d
P-S-R	1.37 b	20.42 a	20.6 ab	36.29 a	22.65 d	50.50 bc	0.95 a	0.20 a

Table 3- Pearson correlation coefficients between some measured soil parameters

Soil properties																			
	Porosity	BD	WFPS	Sand	Coarse Silt	Fine Silt	Clay	AWC	SAI	SMAI	MWD s	WSA s	GMDS	CaCO3	OC	EC	PMN	Active C	EMI	BR28	DNA
Porosity (%)	1.00																				
BD (g cm^{-3})	0.97**	1.00																			
WFPS (%)	0.08	0.10	1.00																		
Sand (%)	0.01	0.07	-0.25	1.00																	
Coars Silt (%)	0.15	0.10	-0.05	-0.53**	1.00																
Fine Silt (%)	-0.23	-0.27	0.41**	-0.77**	-0.04	1.00															
Clay (%)	0.19	0.14	0.05	-0.81**	0.39**	0.44**	1.00														
AWC (g g^{-1})	-0.21	-0.26	0.39**	-0.90**	0.41**	0.84**	0.58**	1.00													
SAI (%)[1]	-0.17	-0.17	-0.06	0.02	-0.21	0.15	-0.07	0.05	1.00												
SmaI (%)[2]	-0.38**	-0.38**	0.15	0.09	-0.34*	0.28	-0.35*	0.09	0.69**	1.00											
MWD s[3]	-0.21	-0.22	0.28	0.10	-0.12	0.16	-0.40**	0.10	-0.15	0.51**	1.00										
WSAs (%)[4]	-0.07	-0.06	0.18	-0.06	0.03	0.15	-0.13	0.20	-0.20	0.31*	0.70**	1.00									
GMDS (%)[5]	-0.05	-0.06	0.22	-0.04	-0.03	0.20	-0.18	0.21	-0.15	0.41**	0.87**	0.85**	1.00								
CaCO3 (%)	-0.49**	-0.49**	0.45**	-0.14	0.03	0.30*	-0.15	0.41**	0.01	0.33*	0.34*	0.10	0.12	1.00							
OC (%)	-0.44**	-0.41**	0.43**	-0.08	-0.13	0.33*	-0.18	0.46**	0.06	0.21	0.22	0.20	0.19	0.68**	1.00						
EC 1:5 (dS m^{-1})	-0.46**	-0.45**	0.04	-0.07	-0.29*	0.39**	-0.16	0.24	0.24	0.40**	0.13	0.07	-0.06	0.59**	0.48**	1.00					
PMN (mg g^{-1} week)[6]	0.05	0.09	0.49**	-0.34*	-0.09	0.41**	0.32*	0.43**	0.10	0.08	-0.02	0.20	0.17	0.12	0.39**	-0.08	1.00				
Active C (mg kg-[1])	-0.67**	-0.67**	0.28	-0.20	-0.05	0.44**	-0.14	0.49**	0.14	0.47**	0.34*	0.21	0.25	0.74**	0.64**	0.54**	-0.04	1.00			
EMI (QBS-ar)[7]	0.18	0.23	0.17	0.30*	-0.12	-0.30*	-0.17	-0.45**	-0.34*	-0.14	0.09	-0.01	-0.07	-0.04	-0.25	-0.10	-0.05	-0.37**	1.00		
BR 28 (µg C-CO_2 g^{-1} h^{-1})[8]	-0.52**	-0.49**	0.16	-0.15	-0.20	0.44**	-0.10	0.33*	0.15	0.45**	0.34*	0.21	0.23	0.49**	0.41**	0.56**	0.29*	0.50**	-0.09	1.00	
ng DNA. D.s.	-0.18	-0.15	0.73**	-0.08	-0.18	0.31*	-0.10	0.27	-0.18	0.17	0.34*	0.25	0.27	0.47**	0.59**	0.21	0.53**	0.32*	0.37**	0.38**	1.00

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-taile)

[1] Stable aggregates index
[2] Stable macroaggregates index
[3] Mean weight diameter of slaked
[4] Water stable of aggregates of slaked
[5] Geometric mean diameter of slaked
[6] Potentially mineralizable nitrogen
[7] Soil biological quality index
[8] Basal respiration at the 28thday of incubation

Soil aggregate stability was assumed a major factor for assessing soil quality. Aggregate stability is used as an indicator of soil structure, which affects many processes including biological activity, infiltration, aeration, and nutrient cycling (Karlen et al., 2006). Tables 4 to 7 present the results of aggregate size stability distribution (see chapter 2), under four rotation systems in the months of April, June, August and October respectively. Our results revealed that the aggregate size stability distribution was significantly influenced by the rotation systems and soil moisture condition. The amount of large macroaggregates (> 2000 μm) in field-moist soil (April) had the following order: F-R > S-R-R = P-S-R > R-R-R. The almost 10.66 % of the soil dry weight was present as stable large macroaggregates under F-R, 1.52% under S-R-R, 1.71% under P-S-R , and 0.62 % under R-R-R (see table 4). Moreover, F-R showed the highest stable large macroaggregates in the months of August and October as well. The higher stable large macroaggregates of F-R rotation in the months of April and October as compared to other systems may have been due to the greater levels of organic carbon in this rotation. There were no significant differences in the distribution of small macroaggregates (250-2000 μm) under the cropping systems in April and August, while P-S-R rotation showed significant differences in both early period of waterlogging and drained soil condition. Furthermore, P-S-R rotation demonstrated the highest microaggregate values across the rice growing season. The amount of unstable macroaggregates (> 250 μm) in field moist condition followed the order; P-S-R > R-R-R = F-R = S-R-R. These results are consistent with findings of Martens (2000), who concluded that the soil instability following soybean production is likely due to the deleterious effects of soya on soil structure. It is assumed that limited return of soybean biomass to the soil and its residue biochemistry, including low concentration of phenols lead to a decrease in aggregate stability. While, from table 7 (drained soil condition) we observed that 11.91% of the soil dry

Table 4- The aggregate-size stability distribution under four different rotation systems in the month of April. Values are represented as percentages of soil dry weight (on a sand-free basis) in each size fraction. Different letters demonstrate differences (P = 0.05) between rotation systems within size classes.

	Water pretreatments			Aggregate-size stability distribution			
Size fraction µm	Slaked (%)	Capillary-wetted (%)	Subsequent- slaked (%)		Stable (%)	Unstable (%)	Gains (%)
R-R-R							
>2000	0.62	3.14			0.62 c	2.52 a	
250-2000	23.99 (73.43)	18.07 (63.14)	14.32		14.32 a	3.74 b	9.67
53-250	12.46 (22.29)	11.67 (30.38)			12.04 b		0.79
<53	3.67 (3.67)	3.33 (3.33)			3.33 b		0.33
Total	40.74 (100)	36.22 (100)		Ts = 30.31			
				T = TS+TU = 36.57		TU = 6.26 b	TG = 10.79
S-R-R							
>2000	1.52	2.10			1.52 b	0.57 a	
250-2000	23.03 (68.81)	14.28 (61.29)	13.19		13.19 a	1.09 b	9.84
53-250	11.14 (23.33)	10.49 (32.52)			10.49 b		0.64
<53	6.33 (6.33)	4.10 (4.10)			4.10 b		2.24
Total	42.02 (100)	30.96 (100)		Ts = 29.30			
				T = TS+TU = 30.96		TU = 1.66 b	TG = 12.73
F-R							
>2000	10.66 (32.81)	11.05 (30.05)			10.66 a	0.39 a	
250-2000	22.58 (50.62)	19.38 (52.95)	17.08		17.08 a	2.31 b	5.50
53-250	5.43 (11.81)	2.47 (11.38)			2.47 c		2.96
<53	4.76 (4.76)	2.83 (5.62)			2.83 ab		1.93
Total	43.43 (100)	35.74 (100)					
				TS = 33.04			TG = 10.39
P-S-R				T = TS+TU = 35.74			
>2000	1.71	2.19			1.71 b	0.47 a	22.83
250-2000	41.90 (59.86)	35.25 (59.86)	19.07		19.07 a	16.17 a	0.61
53-250	21.86 (30.43)	21.25 (30.05)			21.25 a		0.10
<53	8 (8)	7.90 (7.90)			7.90 a		22.83
Total	73.48 (100)	66.59 (100)		TS = 49.94			
				T = TS+TU = 66.59		TU = 16.6 a	TG = 23.54

Table 5- The aggregate-size stability distribution under four different rotation systems in the month of June. Values are represented as percentages of dry weight of soil (on a sand-free basis) in each size fraction. Different letters demonstrate differences (P = 0.05) between rotation systems within size classes.

	Water pretreatments			Aggregate-size stability distribution			
Size fraction µm	Slaked (%)	Capillary-wetted (%)	Subsequent- slaked (%)		Stable (%)	Unstable (%)	Gains (%)
R-R-R							
>2000	1.43	4.19			1.43 c	2.76 a	
250-2000	16.61 (57.29)	14.99 (57.67)	13.82		13.82 b	1.18 ab	2.79
53-250	10.48 (31.57)	8.05 (28.67)			8.05 b		2.43
<53	9.71 (9.71)	9.48 (9.48)			9.48 a		0.24
Total	38.23 (100)	36.71 (100)		Ts = 32.78			
				T = TS+TU = 36.71		TU = 3.94 a	TG = 5.46
S-R-R							
>2000	3.07	5.81			3.07 b	2.74 a	
250-2000	23.41 (56.86)	19.87 (59.52)	15.38		15.38 b	4.49 ab	10.46
53-250	9.98 (27.38)	5.69 (25.10)			5.69 b		3.86
<53	11.71 (11.71)	9.57 (9.57)			9.57 a		1.79
Total	48.18 (100)	40.94(100)		Ts = 33.72			
				T = TS+TU = 16.11		TU = 7.23 a	TG = 16.11
F-R							
>2000	1.00	2.00			1.00 c	1.00 a	
250-2000	10.52 (60.52)	9.35 (66.71)	8.94		8.94 b	0.41 c	1.57
53-250	7.45 (27.21)	5.51 (22.10)			5.51b		1.94
<53	10.76 (10.76)	9.19 (9.19)			9.19 a		1.57
Total	29.72 (100)	26.05 (100)		TS = 24.64			
				T = TS+TU = 26.05		TU = 1.41 a	TG = 5.08
P-S-R							
>2000	5.43	7.33			5.43 a	1.90 a	
250-2000	38.30 (56.95)	39.39 (55.95)	32.90		32.90 a	6.49 a	5.39
53-250	18.10 (26.10)	15.51(25.30)			15.51 a		2.59
<53	11.52 (11.52)	11.42 (11.42)			11.42 a		0.10
Total	73.35 (100)	73.66 (100)		TS = 65.26			
				T = TS+TU = 73.66		TU = 8.39 a	TG = 8.09

Table 6- The aggregate-size stability distribution under four different rotation systems in the month of August. Values are represented as percentages of dry weight of soil (on a sand-free basis) in each size fraction. Different letters demonstrate differences (P = 0.05) between rotation systems within size classes.

	Water pretreatments			Aggregate-size stability distribution			
Size fraction µm	Slaked (%)	Capillary-wetted (%)	Subsequent- slaked (%)		Stable (%)	Unstable (%)	Gains (%)
R-R-R							
>2000	2.38	2.57			2.38 b	0.19 a	
250-2000	23.27 (75.81)	24.92 (72.05)	12.27		12.27 a	12.65 a	11.00
53-250	5.73 (16.29)	4.71 (21.29)			4.71 b		1.01
<53	5.52 (5.52)	4.10 (4.1)			4.10 a		1.43
Total	36.90 (100)	36.30 (100)		Ts = 23.46			
				T = TS+TU = 36.30		TU = 12.84 a	TG = 13.44
S-R-R							
>2000	1.38	3.33			1.38 b	1.95 a	
250-2000	15.06 (61.29)	16.01 (72.81)	14.10		14.10 a	1.91 b	0.95
53-250	7.35 (26.62)	6.26 (19.76)			6.26 b		1.09
<53	10.71 (10.71)	4.10 (4.10)			4.10 a		6.62
Total	34.50 (100)	29.70 (100)		Ts = 25.84			
				T = TS+TU = 29.70		TU = 3.86 a	TG =8.66
F-R							
>2000	5.00	8.39			5.00 a	3.39 a	
250-2000	18.12 (46.31)	19.08 (62.28)	17.76		17.77 a	1.32 b	0.36
53-250	13.14 (37.07)	8.51 (20.38)			8.51 b		4.63
<53	11.62 (11.62)	8.95 (8.95)			8.95 a		2.67
Total	47.88 (100)	44.94 (100)		TS = 40.23			
				T = TS+TU = 44.94		TU = 4.71 a	TG = 7.65
P-S-R							
>2000	2.43	3.52			2.43 b	1.10 a	
250-2000	25.09 (38.23)	34.98 (59.52)	20.03		20.03 a	14.95 a	5.06
53-250	34.82 (47.85)	21.47 (31)			21.24 a		13.35
<53	11.48 (11.47)	5.88 (5.95)			5.88 a		5.60
Total	73.82 (100)	65.85 (100)		TS = 49.58			
				T = TS+TU =65.63		TU = 16.05 a	TG = 24.01

Table 7- The aggregate-size stability distribution under four different rotation systems in the month of October. Values are represented as percentages of dry weight of soil (on a sand-free basis) in each size fraction. Different letters demonstrate differences (P = 0.05) between rotation systems within size classes.

	Water pretreatments			Aggregate-size stability distribution			
Size fraction µm	Slaked (%)	Capillary-wetted (%)	Subsequent- slaked (%)		Stable (%)	Unstable (%)	Gains (%)
R-R-R							
>2000	1.25	1.97			1.25 c	0.72 a	
250-2000	21.44 (61.91)	15.20 (56.79)	12.15		12.15 b	3.04 b	9.28
53-250	13.73 (30.31)	9.21 (35.31)			9.21 ab		4.52
<53	6.48 (6.48)	5.33 (5.33)			5.33 b		1.14
Total	42.88 (100)	31.71 (100)		Ts = 27.94			
				T = TS+TU = 31.71		TU = 3.77b	TG = 14.94
S-R-R							
>2000	3.59	7.24			3.59 b	3.65 a	
250-2000	27.95 (69.89)	16.27 (61.26)			13.83 b	2.44 b	14.12
53-250	12.58 (20.08)	9.75 (24.19)			9.75 ab		2.83
<53	6.45 (6.45)	4.66 (4.66)			4.66 b		1.79
Total	50.58 (100)	37.92 (100)		Ts = 31.83			
				T = TS+TU = 37.92		TU = 6.09 b	TG = 18.57
F-R							
>2000	7.87	9.26			7.87 a	1.39 a	
250-2000	13.21 (55.04)	12.60 (53.86)	2.07		2.07 c	10.52 a	11.14
53-250	7.04 (14.70)	5.55 (16.62)			5.55 b		1.50
<53	3.70 (3.70)	2.43 (2.43)			2.43 c		1.27
Total	31.82 (100)	29.84 (100)		TS = 17.92			
				T = TS+TU = 29.84		TU = 11.92 a	TG = 13.90
P-S-R							
>2000	2.19	3.47			2.19 c	1.28 a	
250-2000	40.13 (61.43)	39.31 (57.92)			34.76 a	4.55 b	4.53
53-250	15.46 (25.57)	14.15 (28.04)			14.15 a		0.85
<53	10.81(10.81)	10.57 (10.57)			10.57 a		0.10
Total	68.59 (100)	67.50 (100)		TS = 61.67			
				T = TS+TU = 67.50		TU = 5.83 b	TG = 5.48

weight was existing as unstable macroaggregates under F-R rotation, 6.08 % under S-R-R rotation, 5.83% under P-S-R, and 3.76 % under R-R-R rotation.

Table 8 illustrates some of the soil stability indices that have been used for assessing overall soil health of the study area under various cropping patterns through the growing season. The SmaI shows a clear trend across the four rotation systems. Stable macroaggregate index differed at field-moist soil (in the month of April) in the order F-R > S-R-R > R-R-R > P-S-R. It is noteworthy to point out that the approximately 70% of the dry weight of the soil under F-R rotation (in the month of April) was stable macroaggregates while only 26% of the soil under the P-S-R rotation consisted of stable macroaggregates. Although the values for stable macroaggregates index (SMAI) are significantly different for R-R-R and S-R-R rotation in the month of April; the values of water stable aggregates applying the capillary wetted pretreatment are not different. It depends on the amount of aggregates (> 250 µm) that survive slaking for R-R-R and S-R-R, which are not significantly different; $S_{1S}+S_{2S}$ is equal to 24.61 and 24.55 for R-R-R and S-R-R, respectively (see Table 4).

The values of stable aggregates (SAI), stable macroaggregates (SmaI), and water stable aggregates of slaked (WSAs) are not significantly different for F-R and P-S-R in the late period of waterlogging. This is due to the amounts of stable macroaggregates (i.e. large and small macroaggregates) which are not significantly different; S1 + S2 is equal to 22.77 and 22.46 for F-R and P-S-R respectively. In addition, The amount of aggregates (> 250 µm) which survived slaking for F-R and P-S-R in August are not significantly different; 23.13 and 27.52 % for F-R and P-S-R respectively. No clear trend (i.e. statistical differences) among rotation systems were observed for SAI and SMAI in the months of June and August.

Table 8- Mean values of the stable aggregates index (SAI), stable macroaggregates index (SmaI), water stable aggregates of slaked (WSAs), water stable aggregates of capillary-wetted (WSAcw), mean weight diameter of slaked (MWDs), mean weight diameter of capillary-wetted (MWDcw), geometric mean diameter of slaked (GMDs), and capillary-wetted (GMDcw) soils under four different rotation systems over the growing season. Same lowercase letters in columns indicate that the soil stability index dose not differ between rotation systems at P = 0.05, ANOVA with Duncan's multiple-range test.

Cropping patterns	**Soil physical properties**							
April	**Indices of soil stability**							
	SAI (%)	**SmaI (%)**	**WSAs (%)**	**WSAcw (%)**	**MWDs (mm)**	**MWDcw (mm)**	**GMDs (mm)**	**GMDcw (mm)**
R-R-R	80.45a	34.38 bc	60.49 ab	57.72 b	0.78 b	1 b	0.70 b	0.71c
S-R-R	92.41 a	42.42 b	45.14 b	53.32 b	0.84 b	0.92 b	0.67 b	0.67 b
F-R	92.22 a	69.65 a	76.66 a	85.27 a	1.83 a	2.17 a	0.92 a	1.06 a
P-S-R	68.78 a	26.78 c	59.32 ab	56.11 b	0.92 b	0.81 b	0.69 b	0.67 b
June								
R-R-R	83.73 a	38.187 a	47.47 ab	52.33 ab	0.73 bc	1.071 ab	0.56 bc	0.62ab
S-R-R	75.37 a	40.65 a	55.71 a	62.62 a	0.91 ab	1.28 a	0.62 ab	0.69 a
F-R	90.61 a	38.84 a	38.90 b	43.75 b	0.61 c	0.83 b	0.48 c	0.52 b
P-S-R	85.75 a	46.02 a	59.56 a	63.39 a	0.99 a	1.13 ab	0.68 a	0.72 a
August								
R-R-R	60.52 a	35.16 a	69.52 a	75.66 a	1.06 a	1.14 ab	0.75 a	0.82 a
S-R-R	83.68a	43.76 a	48.22 b	64.82 a	0.73 b	1.19 ab	0.54 b	0.75 a
F-R	85.16 a	47.60 a	48.03 b	61.24 a	0.99 a	1.45 a	0.59 b	0.73 a
P-S-R	70.82 a	30.29 a	37.34 b	59.10 a	0.62 b	0.92 b	0.55 b	1.04 a
October								
R-R-R	85.34 a	39.17 a	53.32 b	55.44 b	0.76 c	0.90 c	0.60 c	0.65 c
S-R-R	78.36 a	41.12 a	62.63 a	61.62 ab	1.02 b	1.48 b	0.72 b	0.77 b
F-R	58.30 b	40.51 a	66.25 a	73.07 a	1.74 a	2.04 a	0.84 a	0.95 a
P-S-R	59.22 a	46.10 a	61.70 ab	63.38 ab	0.85 bc	0.94 c	0.68 bc	0.70 bc

3.2. Effect of different cropping patterns on soil chemical properties

Descriptive statistics of soil chemical properties, including pH, organic matter content, total carbonates, electrical conductivity, extractable phosphorous, cation exchange capacity, of 48 soil samples are given in Table 9. A wide range of values was found for most of the determined parameters. The chemical soil properties of four different rotation systems over the growth period of rice are illustrated in (Fig. 16). The pH value varied in a rather narrow range (7.2 to 8.54, i.e. neutral to moderately alkaline), with a small seasonal decrease in all rotations, and it was also flooding condition dependent. The overall effect of submergence makes the pH values to converge to neutral (Zheng and Zhang, 2011; McBride, 1994).Yet, when an aerobic soil is submerged, its pH decreases during the first few days (Ponnamperuma, 1972), reaches a minimum (Fig. 14a), until a fairly stable value is attained few weeks later.

The average organic matter content of all soil samples was 29.49 g SOM kg^{-1}, ranging from 14.22 to 64.28 g SOM kg^{-1}, with generally high coefficients of variation. Among the four rotations, S-R-R had the highest (64.28 g kg^{-1}) and the lowest (14.22 g kg^{-1}) organic matter content, and P-S-R had the lowest CV (14.98, with SOM ranging from 17.90 to 33.80 g kg^{-1}) (Fig. 14b).

Total Carbonates had a wide range (2.05-34.88%), with high variance, in relation to pH values and to the parent material, which was derived from alluvial deposits of the Adige river, with mixed mineralogy (Mozzi, 2005).

The wide range of particle size (see table 1) is likely responsible for the recorded differences in CEC, irrespective of the rotation system (Table 9). In fact, clay and organic matter, due to their electrostatic surface charges and large specific surface area, have an important role in soil CEC. The cation holding capacity is related to the different clay types and clay-blends

present in soil, and is very dependent on the proportion of clay and organic matter in the soil (Havlin et al. 2009). According to Mirkhani et al., (2005), CEC of soils ranges from less than 1 $cmol^{+}kg^{-1}$ in sandy soils with low organic matter to more than 25 $cmol^{+}kg^{-1}$ in clay soils with high organic matter. The CEC in the examined samples ranged from 11.57- 41.27 $cmol^{+}$ kg^{-1}; our results showed that, despite decreasing in pH along with the soil flooding and low clay content, CEC is likely increased due to the increase of organic matter in August and October in F-R and S-R-R rotations respectively (Fig. 14c).

Extractable phosphorous of soil samples varied from 170.87 to 43.02 mg kg^{-1}, with decreasing P levels from R –R –R to P –S –R as shown in Table 12. The highest P level (170.87 mg kg^{-1}) was found in R –R –R, and the lowest (43.02 mg kg^{-1}) in S-R-R, were in both April and August, consistently with enhanced P solubility during waterlogging (Chisci, 2009).

The soil electrical conductivity ranged from 0.97 to 2.96 dS m^{-1}, the latter being above the salinity threshold reported by FAO (1976). Grattan et al. (2002) have indicated that rice is moderately sensitive to salinity when it exceeds 2000 µS cm^{-1}: the recorded value, therefore, can substantially reduce rice yield.

3.2.1. Effects of soil characteristics on the contents of trace, macro- and microelements in soil

Table 10 summarizes the total concentrations of Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, P , Pb, Sb, Si, Sn, Sr, Ti, TI, V and Zn from the investigated area. There was a distinct difference among the four rotation systems in the total metal contents. Pea-Soya-Rice rotation had the highest total contents of Al, As, Ba, Be, Cd, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sn, Co and V, and Soya-Rice-Rice had the lowest values of these metals except for As, Mn and Ni. Otherwise, Rice-Rice-Rice rotation had the highest TI, Ti and Sb contents. We have calculated the

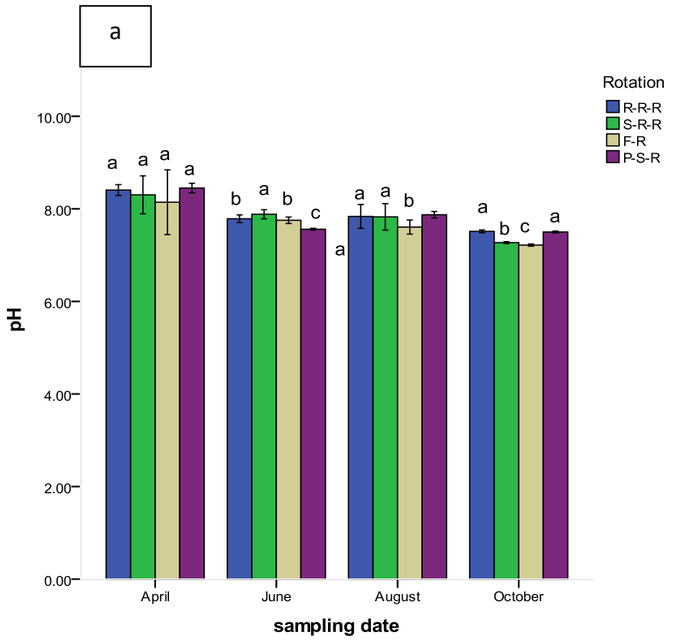
Table 9- Descriptive statistics of soil chemical properties

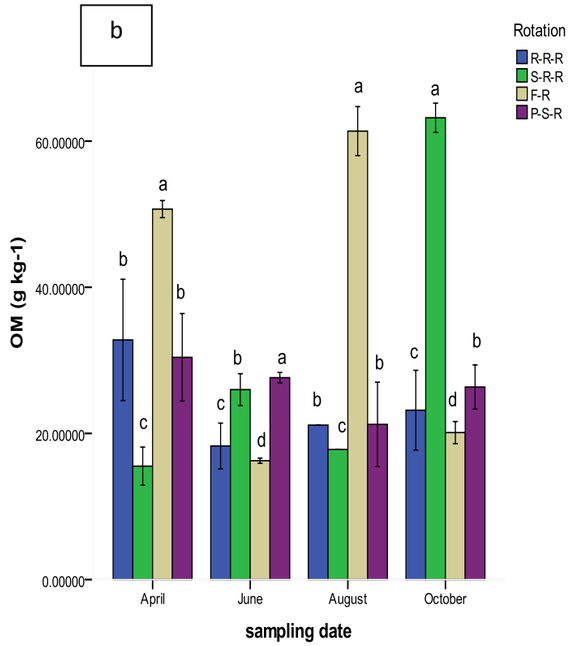
Rotation	**Soil chemical properties**						
		pH In water (1: 2.5)	EC ($dS\ m^{-1}$)	Extractable phosphorous ($mg\ kg^{-1}$)	SOM ($g\ Kg^{-1}$)	$CaCO_3$ (%)	CEC ($cmol^{+}\ kg^{-1}$)
R-R-R	Mean	7.88	1.37	132.82	23.84	3.817	17.360
	Max[1]	8.45	1.63	170.87	37.40	5.69	20.27
	Min[2]	7.50	1.02	92.08	16.48	2.05	15.21
	CV [3](%)	4.37	18.44	25.46	25.61	33.75	8.23
S-R-R	Mean	7.82	2.09	98.13	30.62	15.63	22.32
	Max	8.51	2.96	157.27	64.28	23.08	35.20
	Min	7.26	1.33	43.02	14.22	3.53	11.57
	CV (%)	5.10	24.39	47.39	65.52	48.43	35.36
F-R	Mean	7.68	2.18	72.90	37.11	19.89	25.35
	Max	8.54	2.86	88.99	63.15	34.88	41.27
	Min	7.26	1.33	59.24	16.16	7.53	13.87
	CV (%)	4.93	24.91	13.70	54.47	55.25	45.717
P-S-R	Mean	7.68	1.83	68.93	26.40	13.13	31.30
	Max	8.54	2.96	96.01	33.80	17.65	34.54
	Min	7.49	.97	52.86	17.90	8.90	27.84
	CV (%)	5.02	34.43	19.94	14.98	25.04	7.06

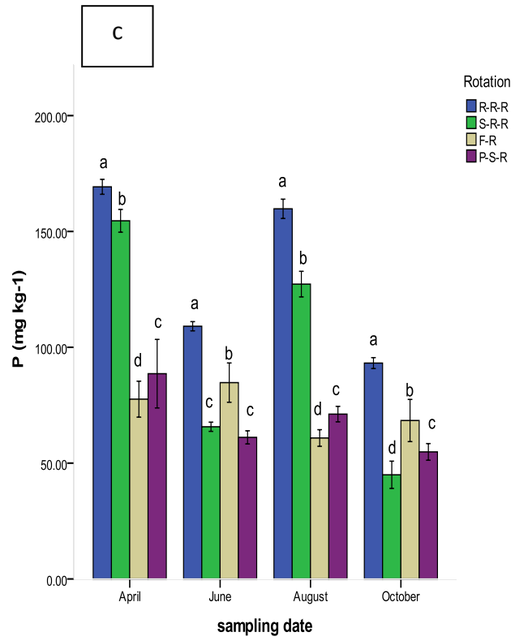
[1] Max, maximum value.
[2] Min, minimum value.
[3] CV, coefficient of variance.

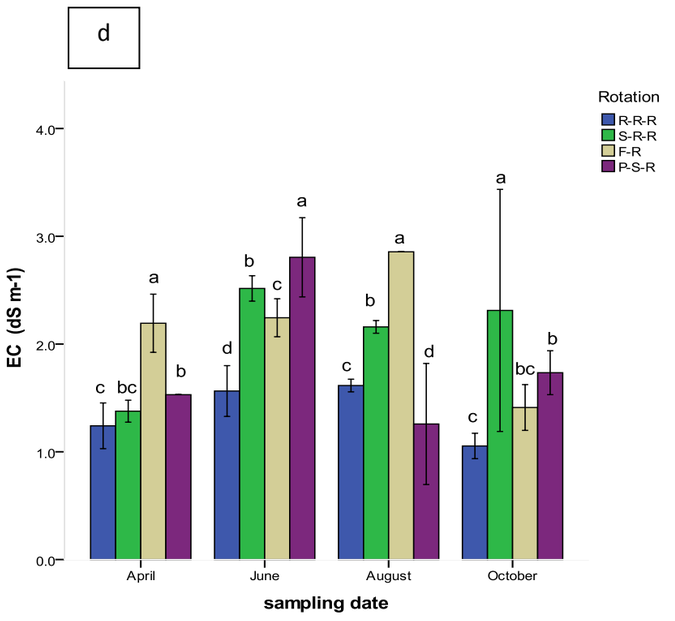
Table 10- Descriptive statistics of metal concentration in soil

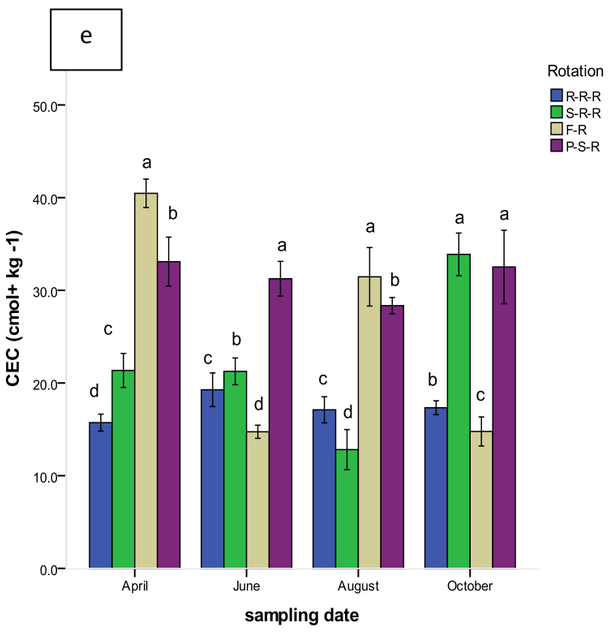
Rotation	*Soil*	*Total element concentration in soil (mg kg^{-1})*																				
		Al	As	Ba	Be	Cd	Co	Cr	Cu	Fe	Li	Mn	Ni	P	Pb	Sb	Si	Sn	Sr	Ti	Tl	Zn	V
R-R-R	Mean	17775.62	6.14	86.59	0.71	0.31	7.32	24.59	14.48	14187.14	29.27	279.16	13.84	960.32	19.70	1.33	133.79	1.71	40.13	1034.98	5.63	65.31	29.75
	Max	20496.65	6.39	103.27	0.79	0.32	7.88	30.11	14.89	15293.65	33.35	324.79	14.19	1010.02	20.65	1.53	173.29	2.05	46.83	1190.38	8.20	69.48	33.74
	Min	15128.71	5.80	73.90	0.63	0.30	6.57	20.91	14.23	13306.93	25.82	213.86	13.54	920.90	19.08	1.19	91.09	1.35	36.44	718.42	3.49	62.97	26.22
	CV (%)	9.55	3.66	11.05	7.06	2.71	5.84	16.58	1.83	4.51	8.25	13.21	1.92	3.25	3.05	9.07	22.91	13.66	9.23	16.85	26.80	3.58	8.05
S-R-R	Mean	13217.01	5.12	78.52	0.50	0.22	4.83	16.13	11.44	9613.53	26.10	245.02	10.66	901.18	14.97	1.14	283.50	1.28	105.99	651.62	2.72	40.09	25.17
	Max	16292.80	5.61	86.58	0.63	0.24	5.27	16.92	12.85	10704.45	30.93	261.01	10.98	1015.27	15.61	1.42	421.38	1.50	137.17	810.70	3.08	43.62	26.89
	Min	11430.82	4.81	62.19	0.39	0.20	4.34	15.45	10.31	8356.33	23.02	222.05	10.31	775.62	14.40	0.95	143.03	1.18	71.28	500.59	2.13	36.95	22.36
	CV (%)	14.12	6.06	11.40	22.52	8.38	8.76	3.08	10.12	11.47	11.80	5.89	2.36	13.88	3.96	16.76	37.99	0.10	31.34	18.35	14.33	7.88	6.73
F-R	Mean	13518.04	5.07	78.06	0.51	0.22	4.94	16.90	11.70	9921.59	26.68	261.26	10.41	1007.73	15.22	0.96	391.88	1.62	104.52	681.07	2.97	40.83	25.62
	Max	14274.45	5.85	92.38	0.55	0.24	5.36	18.56	12.71	10962.15	28.47	278.57	10.58	1182.54	16.19	1.11	743.39	2.13	133.44	754.73	3.23	43.53	28.66
	Min	12130.64	4.58	67.10	0.47	0.20	4.51	15.86	10.41	8864.70	24.11	245.27	10.23	767.95	14.70	0.86	148.26	1.39	76.26	576.98	2.49	37.82	23.09
	CV (%)	6.10	9.26	14.62	7.35	8.03	7.31	5.47	8.54	9.38	6.30	4.55	1.40	13.88	4.07	8.75	66.60	18.67	28.86	9.70	9.43	6.62	9.69
P-S-R	Mean	28388.62	9.76	162.05	0.96	0.35	10.38	41.08	21.62	19355.35	37.32	440.95	27.53	812.68	25.43	1.33	63.52	2.13	96.82	955.81	4.67	70.31	51.52
	Max	30513.83	10.12	176.28	1.03	0.40	10.82	44.19	22.63	20099.73	39.68	463.24	28.23	881.46	27.31	1.49	74.47	2.30	103.56	1028.00	5.22	72.65	54.39
	Min	24216.30	9.33	134.01	0.86	0.30	9.72	36.36	20.69	18103.45	31.90	420.06	26.49	736.59	23.51	1.23	54.47	2.04	92.48	782.92	3.68	66.38	45.53
	CV (%)	8.18	2.68	9.60	5.93	13.06	3.97	6.53	4.30	3.99	8.062	3.26	2.44	8.40	5.60	7.09	12.06	4.63	3.88	10.06	12.18	3.50	6.54











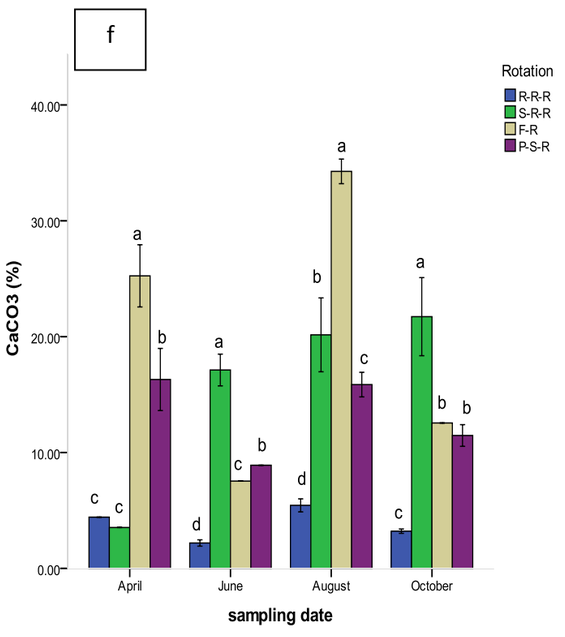


Fig. 14. Chemical properties: a. pH, b. organic matter, c. extractable phosphorus (P), d. electrical conductivity (EC), e. cation exchange capacity, and f. calcium carbonate of soils in various rotation systems: Rice-Rice-Rice, Soya-Rice-Rice, Fallow Rice and Pea-Soya-Rice. Error bars represent ± 2 S.D. from the mean soil chemical indicator value for each rotation. Different lowercase letters indicate significant differences among mean values of each rotation system over the rice-growing season.

Coefficient of Variability (CV) for the investigated metals, to evaluate the possible variability among them.

When CV is less than 10%, it shows low variability; while CV more than 90% shows extensive variability as reported by Zhang et al. (2007). There was a moderate variability in most metal content among the soil samples from each rotation system; for instance, in S-R-R rotation heavy metal content ranged from 11430 to 16292 mg kg^{-1} for Al, and from 8356 to 10704 mg kg^{-1} for Fe, Si ranged between 143 to 421 mg kg^{-1} , Sr was between 71.28 and 137.17 mg kg^{-1} and finally a range of 0.39 to 0.63 mg kg^{-1} for Be, with the coefficient of variation being 14.12%, 11.47, 37.99%, 31.34% 22.52% for Al, Fe, Si, Sr and Be respectively.

The national background values of soils in Veneto region was used as the basis for the threshold values for heavy metal pollution in the soil. Comparing background values to our results, the area is not contaminated by most of the investigated metals (i.e. Al, As, Ba, Be, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sb, Si, Ti, Zn, , and V), whereas there is a high contamination by Li and TI (in all rotation systems). Although Sn (in S-R-R, F-R) concentration is above the permissible limit values according to Italian legislation (D.L. 152/2006) for green and residential areas, the study area is not considered polluted due to the natural geochemical background concentration of this metal (Appendix 5).

Only those elements whose concentrations are above the Italian guidelines will be discussed hereafter. A Two-way analysis of variance (ANOVA) was thus performed on the soil metal concentrations which were above the Italian legislation (D.L. 152/2006) (see Fig. 15), in order to understand the effect of different variables (e.g. rotation systems, time) on the mean metal concentrations, using the LSD test calculated at $P < 0.05$.

The total contents of Li, Sn, Sr and TI, of four different rotation systems over the growth period of rice are shown in Fig. 15. Significant differences ($P < 0.05$) among different sampling times at each rotation were observed. Compared to field-moist soil (April), the P-S-R rotation at waterlogged soil condition (in the month of June) resulted in a decrease in total Li, Sn, and TI contents; afterwards, a gradual increase was observed during growing. Conversely, Sr increased during the first period after plantation, and then decreased sharply, increasing again in the last growing period. In R-R-R rotation, instead, total elements leveled off during the growing season. In S-R-R rotation the total contents of Li decreased dramatically at the early period of waterlogging, while recovered in August (in late waterlogging) and then decreased after rice harvesting. In June, there was a sudden increase for Sr, plummeting in August and then recovered after rice harvesting. There was a decrease for TI and Sn from April to June whereas they remained stable from June to October (Nadimi-Goki et al., 2014).

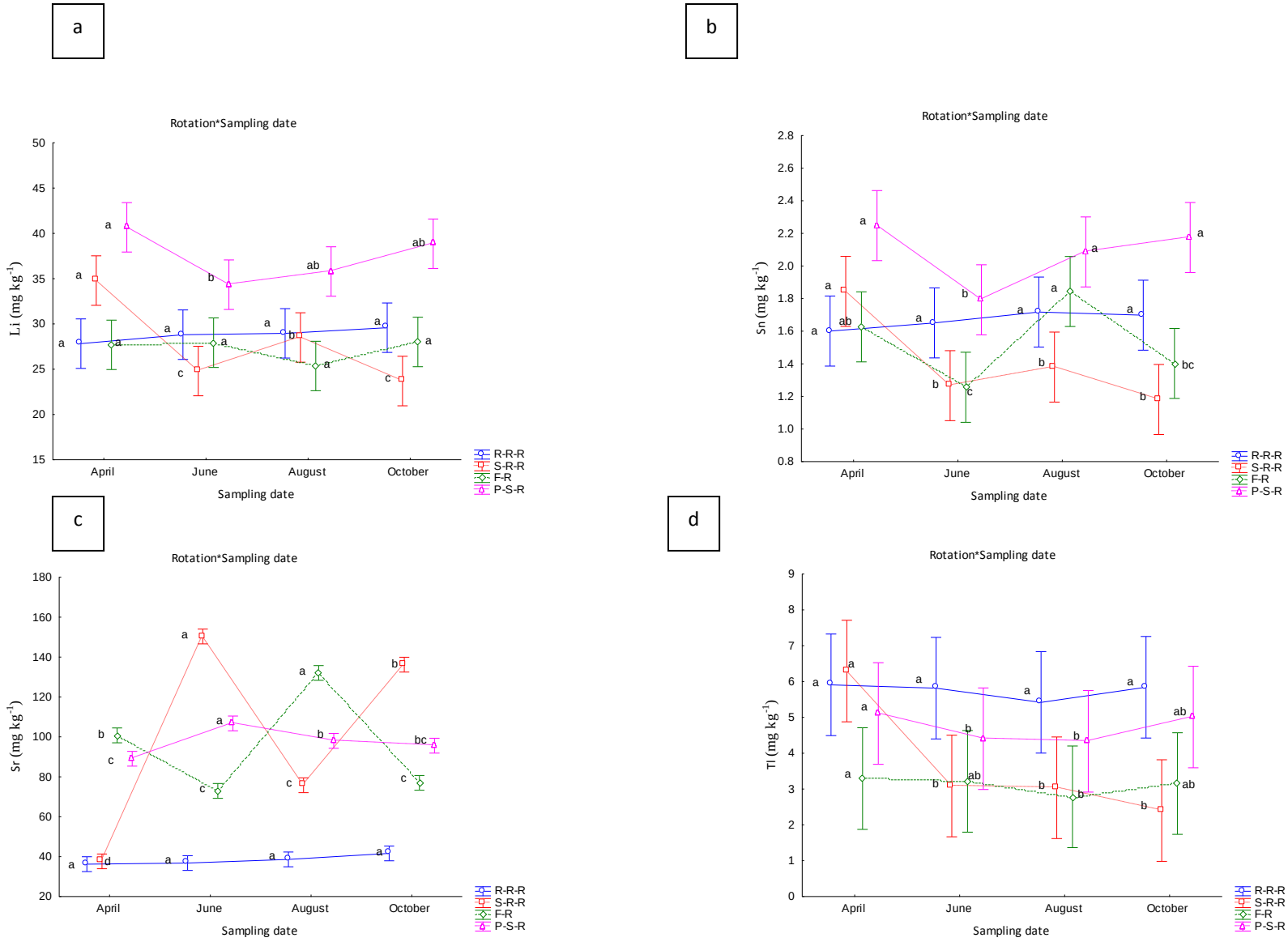


Fig. 15. Total concentration of: a. Li (mg Kg^{-1}), b. Sn (mg Kg^{-1}), c. Sr (mg Kg^{-1}), d. TI (mg Kg^{-1}), of soils in different rotation systems: Rice-Rice-Rice, Soya-Rice-Rice, Fallow-Rice and Pea-Soya-Rice over the growing season (April, June, August, and October). The data are presented as means ± 0.95 confidence intervals from three replicates for each determination. Different lowercase letters indicate significant differences among mean values of different Sampling date, at P = 0.05, two-way ANOVA with LSD test..

Although metals examined do not have the same geochemical behavior, waterlogging determines reductive conditions, and therefore it is likely that this enhances metal mobility to the soil solution; this is true of Sn, Li, and Tl. Conversely, Sr (from carbonate) presents an increase with waterlogging, and this could be ascribed to a major stability of this element in the solid fraction, in reduced conditions. Yet, in these conditions carbonate precipitates from the soil solution, as confirmed by the varying calcium concentrations during the experimental trial (see Fig. 14f).

Pearson correlation coefficients were calculated to determine the relationships between pH, organic matter, clay percentage, CEC, EC, extractable phosphorus and total soil metal contents. The results of correlation analysis showed that the contents of total Ba, Cu, Mn, Ni were strongly affected by CEC and clay %, while total Be, Sb and Ti content were strongly affected by pH and organic matter. There was a significantly negative correlation between the total Be, Fe, Li, Sb, Si, Ti and TI content with soil organic matter; the highest correlation coefficient was found for Ti ($r = - 0.65$, $p < 0.01$) and the lowest was for TI ($r = - 0.52$, $p <0.01$). A significant positive correlation ($p < 0.01$) was observed between most element couples. For example, at $p < 0.01$ Co-Fe: $r = 1$; Al-As: $r = 0.97$; Ni-Cr: $r = 0.97$; TI-Ti: $r = 0.93$; Zn-Cd: $r = 0.92$; Al-Co: $r = 0.98$; and Si-Al: $r = 0.80$, were observed in this study.

The results of this study showed that there is very little correlation between most investigated metals and soil pH (Table 13); yet, at the given pH values (pH >7.2 all over the experimental period) metals have little mobility. Slightly significant correlations ($p < 0.05$) were found with Be, Ti and Zn, possibly because they are slightly mobile in soils and sediments, being attached strongly to particles that contain Al, Fe and Mn (Kabata-Pendias and Mukhrjee, 2007);

antimony showed a more significant positive (R=0.70) correlation ($p<0.01$) with pH, since it is easily adsorbed by soil particles.

Current studies report a well documented negative correlation between soil pH and heavy metal mobility; indeed, metal mobility increases with decreased soil pH (Fanrong et al., 2011; Du Laing et al., 2007; Wang et al., 2006; Badawy et al., 2002), therefore increasing the absorption of heavy metals by plants and thereby posing a threat to human health (Oliver et al., 1996). The role of organic matter on metal behavior in soils has been studied intensively. As widely accepted, heavy metal adsorption onto soil components declined with decreased organic matter content in soils (Antoniadis et al., 2008; Hettiarachchi et al., 2003). Our results demonstrated that there is no correlation ($p < 0.01$) between most transition elements (Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sn, V) and organic matter. Instead As, Fe, Li, Ti, Tl and Zn are negatively correlated, and Si and Sr positively correlated, with organic matter content. It is likely that the rather high soil pH (>7.2) makes it difficult that metals are mobilized from the soil particles. Only Si and Sr present positive correlations with organic matter, being inherited from the parent material. However, these results are not in agreement with findings by Dai et al. (2004), who estimated the Cd, Pb and Zn contents in heavy metal-contaminated soils, and found that the contents of these metals were positively correlated with organic matter.

Table 11- Correlation coefficients between some soil properties and major and trace elements in soil

	Soil properties					Metal concentration in soil																				
	Clay (%)	PH (1: 2.5)	C.E.C ($cmol^{+}\ kg^{-1}$)	P (ppm)	OM ($gr\ Kg^{-1}$)	EC ($dS\ m^{-1}$)	Al	As	Ba	Be	Cd	Co	Cr	Cu	Fe	Li	Mn	Ni	Pb	Sb	Si	Sn	Sr	Ti	Tl	V	Zn
Clay (%)	1.00																										
PH	.30	1.00																									
C.E.C ($cmol\ kg^{-1}$)	.43*	-.19	1.00																								
P (ppm)	-.23	.62**	-.72**	1.00																							
OM ($gr\ Kg^{-1}$)	-.23	-.37	.70**	-.55**	1.00																						
EC ($dS\ m^{-1}$)	-.29	-.05	.40	-.20	.72**	1.00																					
Al	.82**	.32	.28	-.11	-.45*	-.46*	1.00																				
As	.86**	.31	.34	-.19	-.40*	-.46*	.97**	1.00																			
Ba	.86**	.24	.54**	-.33	-.16	-.24	.95**	.94**	1.00																		
Be	.74**	.43*	.08	.08	-.60**	-.56**	.97**	.93**	.86**	1.00																	
Cd	.58**	.26	.22	.04	-.40	-.50*	.85**	.81**	.76**	.85**	1.00																
Co	.77**	.36	.22	-.01	-.50*	-.55**	.98**	.96**	.89**	.98**	.90**	1.00															
Cr	.81**	.27	.35	-.21	-.36	-.49*	.97**	.97**	.94**	.94**	.85**	.97**	1.00														
Cu	.82**	.25	.51*	-.26	-.22	-.36	.94**	.96**	.95**	.88**	.86**	.94**	.96**	1.00													
Fe	.76**	.35	.17	.01	-.55**	-.58**	.97**	.95**	.88**	.98**	.90**	1.00**	.96**	.93**	1.00												
Li	.74**	.28	.14	-.06	-.54**	-.47*	.97**	.90**	.89**	.96**	.79**	.92**	.91**	.84**	.93**	1.00											
Mn	.84**	.27	.44*	-.25	-.30	-.35	.96**	.95**	.97**	.90**	.75**	.93**	.94**	.94**	.91**	.91**	1.00										
Ni	.87**	.30	.42*	-.23	-.33	-.39	.97**	.99**	.96**	.92**	.82**	.96**	.97**	.98**	.94**	.89**	.97**	1.00									
Pb	.77**	.28	.38	-.12	-.33	-.45*	.96**	.94**	.92**	.93**	.92**	.98**	.97**	.98**	.97**	.88**	.92**	.95**	1.00								
Sb	.48*	.70**	-.20	.57**	-.61**	-.41*	.56**	.54**	.39	.65**	.59**	.64**	.52**	.48*	.65**	.52**	.45*	.52**	.58**	1.00							
Si	.52**	-.16	.35	-.27	.84**	.80**	-.63**	-.61**	-.41*	-.71**	-.62**	-.69**	-.60**	-.50*	-.72**	-.65**	-.49*	-.57**	-.58**	-.64**	1.00						
Sn	.50*	.39	.33	-.09	-.23	-.20	.82**	.74**	.81**	.80**	.70**	.80**	.80**	.78**	.78**	.80**	.82**	.77**	.81**	.45*	-.29	1.00					
Sr	.18	-.30	.79**	-.76**	.80**	.67**	-.12	-.05	.18	-.32	-.34	-.25	-.08	.03	-.29	-.17	.08	.02	-.14	-.55**	.63**	-.03	1.00				
Ti	.29	.43*	-.25	.51*	-.65**	-.57**	.67**	.53**	.47*	.76**	.73**	.73**	.59**	.51*	.74**	.70**	.54**	.51*	.66**	.69**	-.63**	.64**	-.67**	1.00			
Tl	.21	.29	-.19	.37	-.52**	-.59**	.58**	.46*	.41*	.68**	.69**	.66**	.57**	.47*	.67**	.61**	.47*	.43*	.63**	.62**	-.55**	.61**	-.62**	.93**	1.00		
V	.85**	.27	.51*	-.29	-.22	-.31	.97**	.96**	.99**	.89**	.80**	.93**	.97**	.97**	.91**	.90**	.98**	.98**	.95**	.45*	-.47*	.82**	.11	.51*	.45*	1.00	
Zn	.57**	.41*	.01	.24	-.59**	-.66**	.85**	.81**	.69**	.91**	.92**	.93**	.85**	.82**	.93**	.78**	.73**	.80**	.90**	.71**	-.73**	.69**	-.53**	.84**	.78**	.75**	1.00

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Yet, it is a major contributor to the ability of soils for maintaining heavy metals in an hosphate n form. Moreover, organic matter also provides organic chemicals to the soil solution that can serve as chelates and enhance the availability of metal to plants (Fanrong et al., 2011; McCauley et al., 2009).

3.3. Metal concentration in rice (root, stem, leaf, grain)

The concentrations of Al, As, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, P, Pb, Sb, Si, Sn, Sr, Ti, TI, V and Zn in rice shoots and roots sampled from four rotations are summarized in Tables 15, 16, 17, and 18 for the roots, stems, leaves and grains respectively.

3.3.1. Metals accumulation in rice roots, shoots and grains

The amount of metal accumulation in plant roots proved to differ among rotation systems (Table 12). Iron resulted as the most abundant, as expected, being an important nutrient for plants, readily available in reduced field conditions (Becker and Asch, 2005). Elements with geochemical affinity to iron (Cr, Mn, Ni) follow it in the abundance sequence, and are followed by chalcophilous elements (Ba, Zn, Cu, Pb); arsenic is generally the less abundant element in the roots with the exception of P-S-R rotation, where As attains the highest amount (up to 172 $mgkg^{-1}$), as indicated by TF (Table 16). Moreover, since As is a known phytotoxic (Kabata-Pendias, 2011; Adriano, 2001), rice plants tend to arrest it in roots (barrier effect). The metal concentrations reported in Tables 12, 13, 14, and 15 show that Fe, Cr, and Pb present amounts above the phytotoxicity threshold (Wahsha et al., 2012b; Kabata-Pendias, 2011; Adriano, 2001) in all rotations and in all different parts of the plant. Further, rice plants (*Oryza sativa* L.) accumulated significant quantities of As and Ba in all the considered parts of the plant except for grain.

Table 12- major and trace elements concentrations in the root of rice grown in the different rotations systems

Rotation	*ROOT*	*Total element concentration in root (mg kg^{-1})*																				
		Al	**As**	**Ba**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Li**	**Mn**	**Ni**	**P**	**Pb**	**Sb**	**Si**	**Sn**	**Sr**	**Ti**	**TI**	**V**	**Zn**
R-R-R	Mean	3923.55	21.80	123.62	1.16	4.93	324.16	28.09	8439.65	7.84	139.88	144.33	1995.23	16.47	2.94	86.82	< DL	35.91	85.30	< DL	14.21	151.58
	Max	3964.04	27.41	143.56	1.53	7.38	366.41	32.84	8621.04	10.16	146.52	165.64	2034.42	24.38	4.61	100.68	-	39.09	89.49	-	16.77	249.53
	Min	3883.10	16.19	103.67	.53	2.48	281.91	23.33	8258.26	5.51	133.23	123.03	1956.04	8.57	.61	72.97	-	32.73	81.12	-	10.65	53.63
	CV (%)	1.08	19.91	15.97	30.09	37.61	13.61	12.49	2.33	23.27	3.86	14.70	2.04	40.75	49.68	15.10	-	6.14	3.51	-	15.61	69.35
S-R-R	Mean	3108.58	49.00	134.68	1.19	4.29	238.06	21.60	9259.25	6.78	175.11	107.31	1849.47	13.54	2.46	60.15	< DL	42.49	64.04	< DL	16.28	191.00
	Max	3720.15	75.08	136.80	2.42	5.64	242.44	26.00	10454.42	9.17	252.69	110.28	1933.42	16.58	4.52	89.50	-	55.92	67.67	-	25.49	287.83
	Min	2497.01	22.92	132.55	.42	2.93	233.67	16.00	8064.08	4.39	97.52	104.35	1765.52	10.50	.52	30.80	-	29.05	60.42	-	7.08	94.18
	CV (%)	21.48	53.96	1.33	57.42	22.74	1.33	17.31	14.12	23.27	47.30	1.94	4.85	15.67	57.67	49.90	-	29.78	3.94	-	49.72	54.39
F-R	Mean	3315.74	46.12	128.38	1.22	4.07	246.15	19.65	9250.59	7.52	169.36	108.62	2106.95	12.49	2.36	63.16	< DL	49.23	75.69	< DL	14.93	98.52
	Max	4655.54	50.62	179.17	2.00	5.77	278.38	23.28	10296.02	12.24	172.21	124.20	2568.93	14.99	3.52	90.19	-	60.58	101.53	-	17.51	141.48
	Min	1975.94	41.62	77.59	.95	2.36	213.92	16.03	8205.17	2.81	166.50	93.04	1644.97	9.98	1.21	36.14	-	37.87	49.85	-	13.34	55.56
	CV (%)	44.20	7.09	41.65	35.51	29.02	13.47	12.83	12.36	46.14	1.19	13.80	23.92	15.00	38.54	43.49	-	21.13	34.58	-	10.40	45.58
P-S-R	Mean	3715.80	87.29	79.87	1.76	4.31	195.77	25.91	12208.78	6.30	234.36	12208.78	5604.97	8.66	2.20	81.58	< DL	26.82	67.86	< DL	16.08	54.49
	Max	4917.72	172.13	132.15	3.71	7.01	302.27	33.43	20993.38	10.47	351.67	133.32	1593.53	13.60	5.14	99.62	-	41.20	97.57	-	26.46	71.62
	Min	2513.87	2.44	27.60	.30	1.61	89.27	18.38	3424.19	2.13	117.05	44.90	902.76	3.71	.27	63.54	-	12.44	38.16	-	6.70	37.37
	CV (%)	35.37	103.99	68.98	92.09	47.89	53.90	24.35	78.80	68.98	53.90	51.92	30.14	42.60	79.21	21.65	-	30.59	44.80	-	57.77	30.59

R-R-R:Rice-Rice-Rice,
S-R-R: Soya-Rice-Rice, Fallow-Rice
P-S-R: Pea-Soya-Rice
MAX: maximum value
Min: minimum value
CV (%): coefficient of variance

Table 13- major and trace elements concentrations in stem of rice grown in the different rotations systems

Rotation	*Stem*	*Total element concentration in stem (mg kg^{-1})*																				
		Al	**As**	**B**	**Ba**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Li**	**Mn**	**Ni**	**P**	**Pb**	**Si**	**Sn**	**Sr**	**Ti**	**TI**	**V**	**Zn**
R-R-R	Mean	185.29	1.75	2.70	16.22	< DL	< DL	34.12	10.42	314.43	1.54	87.48	15.35	1407.55	5.17	63.14	< DL	8.53	5.48	< DL	.56	42.47
	Max	187.29	3.19	4.70	18.22	-	< DL	36.12	12.42	316.43	2.64	89.48	17.35	1409.55	7.17	65.14	-	10.53	7.48	-	.56	44.47
	Min	183.29	.85	.70	14.22	-	< DL	32.12	8.42	312.43	.64	85.48	13.35	1405.55	3.17	61.14	-	6.53	3.48	-	.55	40.47
	CV (%)	.97	64.85	66.16	11.03	-	< DL	5.24	17.17	.57	58.66	2.04	11.66	.13	34.61	2.83	-	20.98	32.65	-	.82	4.21
S-R-R	Mean	192.47	1.59	1.54	16.30	< DL	0.19	20.80	6.67	256.89	1.54	78.39	9.31	1094.30	2.02	51.46	< DL	8.02	5.42	< DL	.38	27.24
	Max	194.47	2.78	2.62	18.30	-	0.20	22.80	8.67	258.89	2.62	80.39	11.31	1096.30	4.02	53.46	-	10.02	7.42	-	.39	29.24
	Min	190.47	.78	.62	14.30	-	0.19	18.80	4.67	254.89	.62	76.39	7.31	1092.30	.02	49.46	-	6.02	3.42	-	.36	25.24
	CV (%)	.93	59.05	58.62	10.98	-	4.198	8.60	26.80	.70	58.62	2.28	19.21	.16	88.65	3.48	-	22.31	32.97	-	3.96	6.57
F-R	Mean	334.39	1.68	2.55	23.09	< DL	< DL	21.42	6.21	430.73	2.11	206.21	9.47	1679.94	2.47	141.72	< DL	7.80	9.87	< DL	1.11	39.89
	Max	336.39	3.04	4.55	25.09	-	-	23.42	8.21	432.73	3.11	208.21	11.47	1681.94	4.47	143.72	-	9.80	11.87	-	2.11	41.89
	Min	332.39	.96	.55	21.09	-	-	19.42	4.21	428.73	1.11	204.21	7.47	1677.94	.47	139.72	-	5.80	7.87	-	.11	37.89
	CV (%)	.53	62.78	70.21	7.75	-	-	8.35	28.81	.42	42.40	.87	18.88	.16	88.65	1.26	-	22.92	18.12	-	80.24	4.48
P-S-R	Mean	1080.64	1.39	2.39	21.84	< DL	< DL	21.77	7.41	881.36	2.66	198.38	99.88	1536.09	2.24	49.94	< DL	5.47	26.71	< DL	2.24	82.59
	Max	1082.66	2.15	4.39	23.84	-	-	23.77	9.41	883.36	3.93	200.38	101.88	1538.09	4.24	51.94	-	7.47	28.71	-	3.24	84.59
	Min	1078.60	.15	.39	19.84	-	-	19.77	5.41	879.36	1.93	196.38	97.88	1534.09	.24	47.94	-	3.47	24.71	-	1.24	80.59
	CV (%)	.17	69.53	74.76	8.19	-	-	8.22	24.14	.20	36.99	.90	1.79	.12	79.91	3.58	-	32.71	6.70	-	39.96	2.17

Table 14- major and trace elements concentrations in leaf of rice grown in the different rotations systems

Rotation	***Leaf***	*Total element concentration in leaf (mg kg^{-1})*																			
		Al	**As**	**Ba**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Li**	**Mn**	**Ni**	**P**	**Pb**	**Sb**	**Si**	**Sn**	**Sr**	**Ti**	**TI**	**V**	**Zn**
R-R-R	Mean	221.95	5.50	31.63	< DL	< DL	8.86	5.12	268.03	1.63	186.28	3.62	863.78	6.78	< DL	86.47	< DL	29.37	5.21	< DL	.57	41.92
	Max	225.17	9.85	39.40	-	-	14.44	7.83	288.00	2.78	236.92	6.97	1292.82	12.73	-	95.31	-	32.98	7.72	-	.62	60.26
	Min	218.73	1.15	23.86	-	-	3.28	2.41	248.06	.63	135.64	.26	434.74	.84	-	77.62	-	25.76	2.70	-	.53	23.58
	CV (%)	1.01	56.96	20.78	-	-	48.64	38.06	7.38	55.72	28.62	64.25	54.16	68.96	-	8.92	-	8.55	35.98	-	6.16	42.90
S-R-R	Mean	307.16	7.61	86.17	< DL	< DL	33.90	4.91	462.44	1.63	255.34	15.26	730.65	2.67	1.02	74.41	< DL	30.32	7.14	< DL	.82	54.79
	Max	429.55	15.70	144.41	-	0.37	36.86	7.07	505.17	2.95	412.12	17.72	928.07	4.85	2.03	85.14	-	38.58	11.42	-	.95	89.09
	Min	184.77	.55	27.93	-	0.32	30.94	2.75	419.71	.86	98.55	12.81	533.23	.49	0.03	63.68	-	22.07	2.86	-	.70	20.49
	CV (%)	42.94	89.62	71.53	-	4.995541	71.53	36.63	9.66	60.49	66.41	12.17	29.30	67.40	69.29775	13.07	-	23.35	43.07	-	16.40	64.66
F-R	Mean	279.98	4.38	51.77	< DL	< DL	37.99	5.66	492.07	1.88	204.99	18.72	1138.69	3.36	1.09	74.41	< DL	30.32	7.14	< DL	.82	54.79
	Max	430.24	8.82	71.51	-	-	63.57	8.43	726.55	3.25	265.53	29.61	1225.53	6.47	2.33	75.49	-	33.77	13.84	-	2.57	48.59
	Min	129.73	.20	32.04	-	-	12.41	2.88	257.59	.54	144.45	7.84	1051.86	.25	0.33	69.21	-	23.02	1.39	-	.45	11.87
	CV (%)	58.01	72.45	37.68	-	-	68.15	35.00	51.76	52.14	31.29	52.84	8.16	64.43	63.75048	3.01	-	14.47	65.12	-	86.51	59.58
P-S-R	Mean	837.52	2.22	28.74	< DL	< DL	40.70	6.34	976.63	2.11	322.45	20.62	783.73	2.99	0.98	77.76	< DL	20.32	15.27	< DL	2.08	48.61
	Max	1497.47	4.68	34.82	-	1.67	65.05	10.82	1626.24	4.60	358.83	35.14	1111.80	5.62	1.52	82.35	-	25.08	29.08	-	4.54	76.30
	Min	177.60	.68	22.66	-	0.20	16.35	1.86	327.03	.60	286.08	6.11	455.66	.36	0.45	73.17	-	15.55	1.47	-	.62	20.92
	CV (%)	86.05	72.28	16.76	-	88.84004	60.31	51.29	72.64	71.89	11.69	67.02	45.58	64.07	45.64429	4.31	-	17.31	85.44	-	82.40	58.01

Table 15- major and trace element concentrations in grain of rice grown in the different rotations systems

Rotation	*Grain*	*Total element concentration in grain (mg kg^{-1})*																			
		Al	As	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Ni	P	Pb	Sb	Si	Sn	Sr	Ti	TI	V	Zn
R-R-R	Mean	97.76	< DL	6.57	< DL	< DL	16.98	4.41	189.48	1.45	49.93	8.17	1646.04	1.95	< DL	85.90	< DL	3.81	3.36	< DL	0.55	22.86
	Max	148.95	-	12.92	-	-	31.71	6.49	301.80	2.47	58.35	15.60	2296.05	4.04	-	106.85	-	7.58	6.50	-	0.55	26.83
	Min	46.56	-	.21	-	-	2.26	2.32	77.15	.24	41.52	1.88	996.04	.04	-	64.95	-	.05	.23	-	0.55	18.89
	CV (%)	55.16	-	77.65	-	-	82.76	40.64	63.79	63.12	14.52	74.73	43.13	83.15	-	24.25	-	69.05	64.72	-	0.01	12.26
S-R-R	Mean	371.46	< DL	10.90	< DL	< DL	51.48	5.48	511.90	1.68	58.91	23.74	1067.08	2.32	1.09	55.59	< DL	4.22	9.10	< DL	1.72	20.79
	Max	722.88	-	20.92	-	-	101.30	9.51	986.52	3.33	89.57	47.35	2091.20	4.66	2.33	74.93	-	9.19	18.03	-	2.72	32.10
	Min	20.07	-	.83	-	-	.83	.83	.83	.23	.83	.83	.83	.66	0.33	.83	-	.83	.83	-	0.72	.83
	CV (%)	103.04	-	82.34	-	-	101.85	54.77	101.22	66.10	59.94	99.90	78.97	68.58	63.39	49.53	-	77.93	85.30	-	58.14	56.92
F-R	Mean	275.65	< DL	10.07	< DL	0.58	24.72	4.40	381.97	1.55	49.01	11.73	1337.77	2.22	1	83.28	< DL	7.08	7.88	< DL	2.09	28.92
	Max	516.68	-	17.84	-	0.59	43.19	6.95	679.90	3.00	60.27	21.40	2325.91	4.47	1.02	110.96	-	13.75	15.45	-	3.08	40.64
	Min	34.61	-	2.31	-	0.58	6.25	1.86	84.05	.27	37.75	2.06	349.62	.12	0.98	55.60	-	.42	.31	-	1.08	17.20
	CV (%)	95.00	-	65.18	-	0.85	73.33	42.83	84.87	64.06	21.02	73.26	80.75	79.35	1.44	33.85	-	76.38	79.20	-	47.93	37.33
P-S-R	Mean	429.21	< DL	9.54	< DL	0.64	21.79	4.96	439.12	1.62	68.04	11.01	955.13	2.46	1.10	79.11	< DL	3.73	8.38	< DL	1.95	25.82
	Max	830.80	-	18.56	-	0.84	39.17	7.94	833.47	3.17	111.33	20.20	1889.53	4.34	2.10	102.74	-	8.48	16.29	-	2.95	40.42
	Min	28.28	-	1.73	-	0.44	2.10	2.10	2.10	.24	2.10	2.10	2.10	.34	0.1	2.10	-	.66	1.64	-	0.95	2.10
	CV (%)	101.82	-	81.99	-	31.04	78.39	41.34	84.87	66.07	69.41	73.13	80.14	58.72	90.8	48.69	-	83.10	79.20	-	51.22	58.70

Zinc concentrations fell in the range of normal values for grains in all rotations, while it was above the normal range in the roots and leaves in all rotations and in stems for all rotations except S-R-R. Nickel and titanium were above the toxicity threshold in the roots in all rotations, whereas significant values of Ni occurred in stems in R-R-R and P-S-R, and Ti in F-R and P-S-R as well. In both leaves and grains, Ni and Ti concentrations were slightly above the toxicity threshold in S-R-R, F-R and P-S-R rotations. All metal concentrations in rice are generally higher in the roots than in the aerial parts, and in the leaves higher than in the stems.

The mean metal (As, Ba, Cr, Cu, Mn, Ni, Pb, Zn, V) concentrations in soils and rice plants are different for each rotation system (Fig. 14). It appears that non essential elements (As, Cr, Ni, Pb) are accumulated in the roots more than in the soil and in the aerial parts, irrespective of the rotation system; it is likely that the root acts as a barrier effect, thereby impeding metal translocation to shoots (Wahsha et al., 2014a). Conversely, essential elements (Cu, Zn) are partly allowed to pass the root barrier and translocate to shoots, until a critical level is attained. It is reported in current literature that the zinc concentrations in cereal grains from worldwide vary between 18 and 33 mg kg^{-1}, being the lowest in rice and highest in oats (Kabata-Pendias and Mukherjee, 2007). Manganese, instead, is concentrated in the soil, but part of it accumulates in the roots, from where it is easily translocated to the aerial parts (see Table 20), and in particular to leaves, irrespective of the rotation.

The highest metal concentrations occurred, in general, in the P–S–R rotation, possibly because of the highest metal amount in the soil, whose texture is finest than the other rotation systems. Indeed, the clay fraction is able to adsorb more metals, and subsequently to release them to plants; this is in agreement with the higher cation exchange capacity recorded at the site of P-S-R- rotation (Nadimi-Goki et al., 2014).

Table 16 - Translocation Factor (TF) from soil to root in paddy field of NE Italy, under the different rotations systems

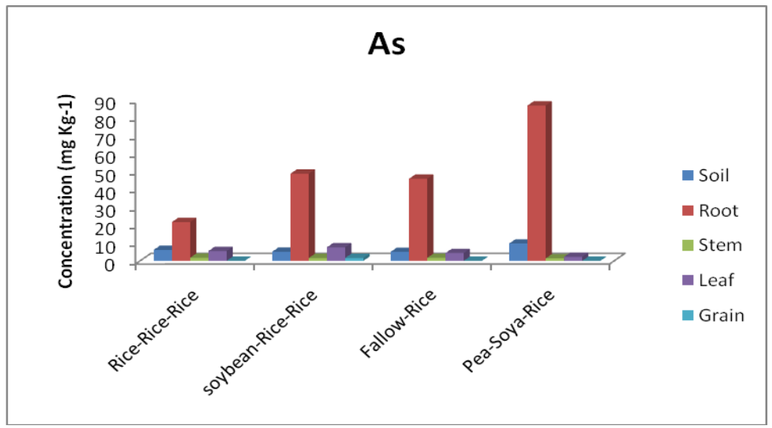
	Translocation factor (TF) Soil to root			
Metals	Rotation			
	R-R-R	S-R-R	F-R	P-S-R
As	3.05	4.64	9.93	0.45
Ba	1.67	1.81	0.9	0.19
Cr	13.49	14.37	12.55	2.27
Cu	1.47	1.5	1.24	0.78
Fe	0.58	0.76	0.9	0.18
Li	0.26	0.25	0.19	0.11
Mn	0.46	0.41	0.65	0.27
Ni	9.06	10.17	9.02	1.74
Pb	1.15	0.87	0.76	0.24
Si	0.47	0.17	0.06	1.4
Sr	0.9	0.71	0.44	0.4
Ti	0.07	0.08	0.08	0.04
Zn	3.74	6.66	3.63	0.58

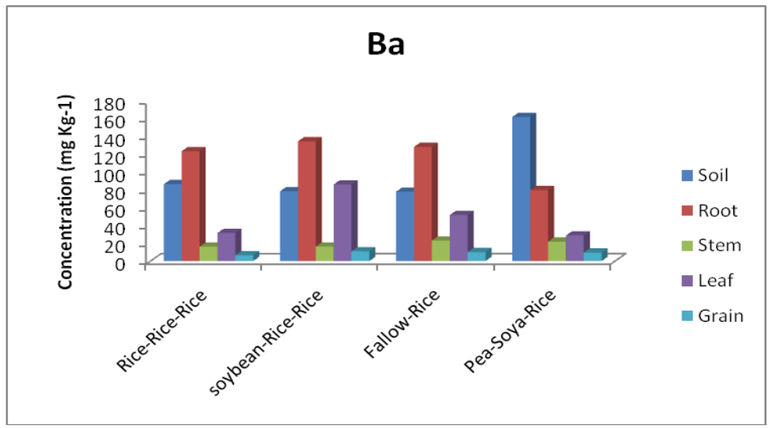
Table 17 - Translocation Factor (TF) from root to leaf in paddy field of NE Italy, under the different rotations systems

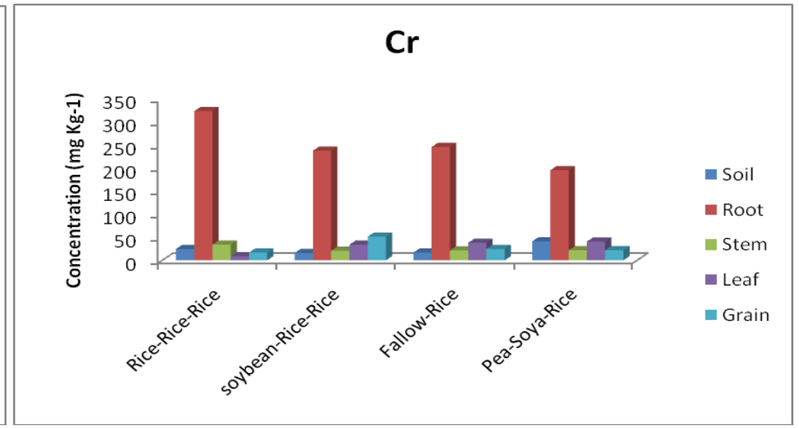
	Translocation factor (TF) root to leaf			
Metals	Rotation			
	R-R-R	S-R-R	F-R	P-S-R
As	0.43	0.06	0.04	0.39
Ba	0.26	0.22	0.43	1.11
Cr	0.04	0.14	0.29	0.69
Cu	0.19	0.21	0.3	0.28
Fe	0.034	0.062	0.088	0.47
Li	0.23	0.23	0.47	0.62
Mn	1.01	1.01	1.54	2.42
Ni	0.04	0.14	0.29	0.71
Pb	0.48	0.23	0.37	0.63
Si	1.24	2.53	1.93	0.77
Sr	0.89	0.68	0.54	0.59
Ti	0.07	0.15	0.23	0.67
Zn	0.1	0.3	0.33	1.89

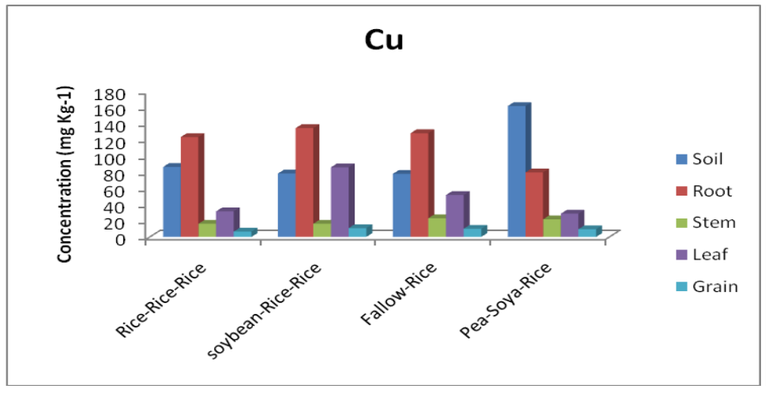
Table 18-
Translocation Factor (TF) from leaf to grain in paddy field of NE Italy, under the different rotations systems

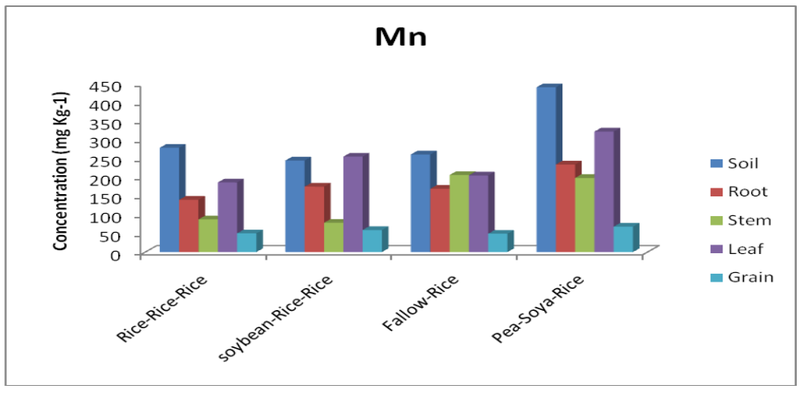
	Translocation factor (TF) leaf to grain			
Metals	Rotation			
	R-R-R	S-R-R	F-R	P-S-R
As	< DL	1.07	< DL	< DL
Ba	0.29	0.63	0.47	0.5
Cr	2.38	3.01	0.66	0.58
Cu	0.74	1.84	0.76	0.67
Fe	1.04	1.95	0.93	0.51
Li	0.87	1.36	0.74	0.66
Mn	0.4	0.87	0.22	0.37
Ni	2.73	3.06	0.7	0.54
Pb	0.19	0.93	0.55	0.65
Si	0.72	0.74	1.48	1.34
Sr	0.07	0.07	0.08	0.08
Ti	0.78	1.7	1.13	0.52
Zn	0.97	0.35	0.83	0.52

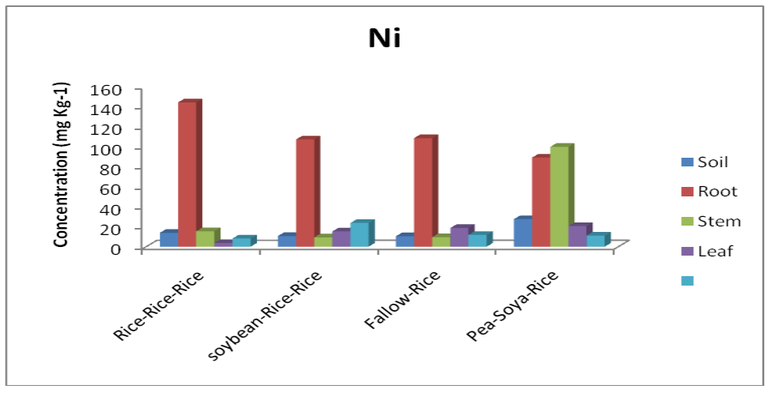


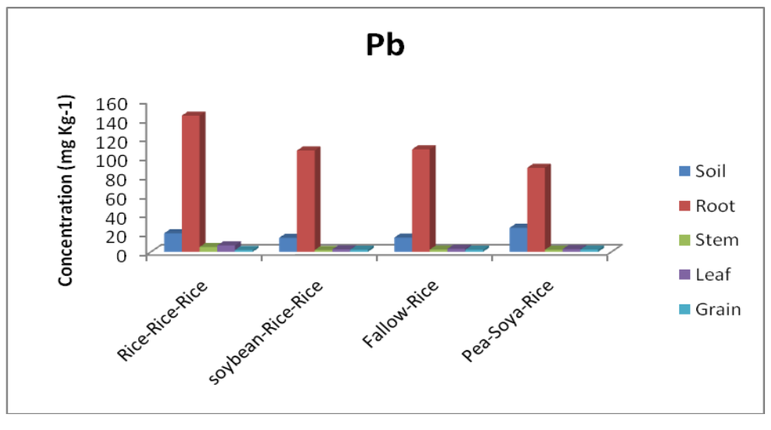


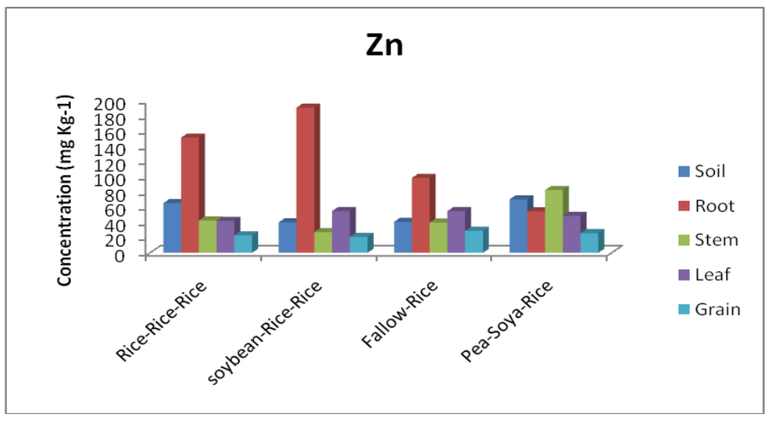












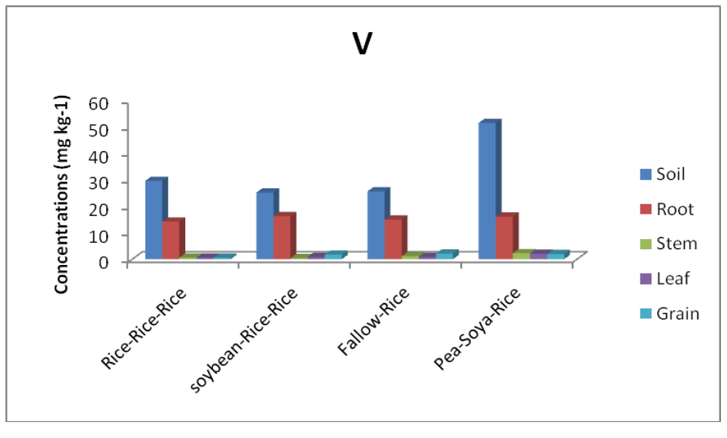


Fig. 16. Accumulations of metals in soil, root, stem, leaf, and grain in Rice-Rice-Rice, Soya-Rice-Rice, Fallow-Rice, and Peas-Soya-Rice rotation systems.

3.4. Translocation Factor:

The translocation factor (TF) or mobilization ratio (Singh et al., 2010; Gupta et al., 2007) was considered to find out relative translocation of metals from the soil to the root (TF_S), root to leaf (TF_R) and leaf to grain (TF_L) for each rotation system over the maturation phase (month of August). TF is defined as ratio of the total element concentration in the aerial parts of the plant to total element concentration in corresponding tissue or soil (Singh et al., 2010).

The most important factors that control the phytoavailability of trace elements include plant absorption ability and soil parameters such as pH and redox potential, texture, organic matter quantity and quality, mineral composition, temperature, water regime, antagonistic and synergistic relations (Wahsha et al., 2012a; Kabata-Pendias, 2004).

Compared to the late period of waterlogging, translocation factor from soil to root (TF_S) for Cr, Cu, Li, Ni, Mn, and Ba in the month of October (drained-soil) resulted in increasing in 132% in the S-R-R, 117% in the R-R-R , 194.7% in the F-R, 131.70% in the F-R, 292% in the P-S-R, and 290% in the F-R rotation respectively (data not shown for the month of October).The results of translocation factor showed that the mobility of individual metal is different within various plant tissues. The metal translocation from soil to root was higher than those from root to shoot. Moreover, when there was a high metal concentration in the soil, metal translocation from soil to root and root to shoot was lower (e.g. excluder plants for Li, Sn, Tl) than that of those metals having low concentration in the soil (e.g. metals are accumulated in roots).

The less translocated element from soil to root is Ti (average TF = 0.067), whereas Cr, Ni, and As (average TF= 10.67, 7.49 and 4.51, respectively) have the highest translocation factor among the elements considered. As it has been mentioned before, the land is not contaminated by Cr, Cu, Ni, Mn, Pb, Ba, Zn and As, but their concentrations in plant tissues (Appendix 5) are

above the normal threshold reported by Kabata-Pendias and Mukhrjee, 2007. Whereas Cu, Mn, and Zn are essential elements for plant growth, As, Ba, Cr, Ni and Pb are non-essential but toxic to plants even at low level, and also to humans through the food chain (Zheng and Zhang, 2011). It may thus be suggested that rice could be useful in contaminated-sites restoration projects, by phytoextraction technique, only in areas contaminated with As, Ba, Zn. Conversely, Cr, Cu and Ni are likely available for phytostabilization. Our findings suggest that rice is an excluder plant for Li, Sn, Tl (i.e. non essential nutrients). Moreover, there is very limited hazard for human population consuming rice crops. It is noteworthy to point out that trace element concentration in plants is highly related to the chemical composition of growth media. Based on this relationship, there is a possibility to identify the origin of food crops that occurred in markets (Szefer and Nriagu, 2006).

According to Liu et al. (2006) rice is susceptible to As accumulation compared to other cereals, given that the baseline levels of As are about 10-fold higher than other cereal grains. It is generally believed that As concentration is much higher in the root than that of the shoot and raw rice grain (Xu et al, 2008; Azizur Rahman et el, 2007; Liu et al, 2006). The high content of As in rice shoots is likely to derive from the xylem transport; by contrast, low accumulation of As in grain is due to the phloem transport.

Lead is one of the most important trace heavy metals due to its serious toxicity even at very low concentrations, which causes harmful effects to humans such as: damaging nervous system, disordering blood and brain (Bini and Wahsha, 2014; Abbasi et al., 2011). In this study, lead transolocation is generally high from soil to roots ($TF \geq 1$), out of P-S-R rotation (previous legume cultivation effect?), while translocation to the aerial parts is rather low ($TF < 1$). Therefore, health hazard for food consumers is low. Si, Mn, Sr and Zn appeared to be the most

translocated among the elements considered, from roots to leave (average TF=1.61, 1.49, 0.67 and 0.69, respectively), while Fe, As, Cu, Cr and Ni had the lowest translocation (average TF < 1).

Calculated translocation factor from leaf to grain showed that As (below DL) and Sr (TF=0.07-0.08) are the least translocated (Table 21). While Cr, Ni, Si, Ti are the most translocated, although not all the rotation systems present the same metal behavior. Indeed, S-R-R- is the most active system in translocating metals from leaves to grains, while P-S-R- is the least active.

3.5. Effect of different cropping patterns on soil Biological properties

3.5.1. Patterns of soil enzyme activities (B-glucosidase, Arylsulfatase, Alkaline and acid phosphatases, Leucine aminopeptidase, Chitinase)

3.5.1.1. *B-glucosidase (βG*)

In paddy soils there is usually a three-month waterlogging period during rice growth which results in enzyme activities that could be different from those of other soil types. Although some research has been done on B-glucosidase in some soil types or ecosystems (Liang et al., 2014; Wallenius et al., 2011; Prieto et al., 2011, Yan et al., 2012), few studies have been conducted in paddy soils (Jinlong et al., 2010; Xiao-Chang and Qin, 2006).

The effects of different rotation systems and sampling periods (different cropping patterns and four sampling dates) on measured enzyme activities were tested by Two-way analysis of variance (ANOVA), and comparisons among treatment means were made using the LSD test calculated at $P < 0.05$.

The activities of B-glucosidase in the plow layers (0-15 cm) of four different rotation systems over the growth period of rice are shown in (Fig. 17). In the waterlogged soil condition

in June, which was at the early period of waterlogging, activity of B-glucosidase in R-R-R and P-S-R rotations increased significantly ($P \leq 0.05$) compared to that of the field-moist condition in April, while B-glucosidase activity was decreased significantly in S-R-R and F-R rotations. The present findings, associated with S-R-R and F-R rotations, seem to be consistent with results reported by Xiao-Chang and Quin (2006) who found a significant decrease ($P \leq 0.05$) of B-glucosidase activity, in waterlogged soil condition, compared to those of the field-moist condition, although it is noteworthy to point out that activity of B-glucosidase in S-R-R and F-R rotations increased with waterlogging time. Activity of B-glucosidase increased significantly *(*$P \leq 0.05$) in the month of October, drained soil condition, compared to that of the field moist soil, except of F-R, in all rotations (Fig. 17).

Pearson linear correlations between parameter couples (i.e. measured soil enzymes, DNA, pH, EC, extractable P, SOC and soil particle size distribution) were conducted using SPSS program at 0.05 probability levels.

In this research, no correlation between organic carbon and B-glucosidase was found, as well as between P and βG (see table 19). The βG activity is often reported to be significantly correlated to SOC, and accounts for 53 to 100% of the variation (r^2) (Jinlong et al., 2010; Acosta-Martínez et al., 2007; Leon et al., 2006; Roldán et al., 2005; Bandick and Dick, 1999; Eivazi and Tabatabai, 1988). There are many exceptions, however, with those studies showing no significant correlation (r^2 from 0.13 to 0.42) between SOC and βG activity (Dodor and Tabatabai, 2005; Acosta-Martínez et al., 2003; Taylor et al., 2002; Bandick and Dick, 1999; Eivazi and Tabatabai, 1988).

Despite low level of organic carbon and coarse texture (i.e. sandy loam), R-R-R rotation had the highest mean level of βG activity. This result disagrees with previously reported

researches that crop rotation systems provide greater amounts of plant residues with different degrees of decomposition that would support higher enzyme activities compared to monoculture continuous system (Moor et al., 2000; Robinson et al., 1996). However, Ekenler and Tabatabai (2003) reported that βG activity is changed not only by soil organic matter content, but also by its quality. Besides R-R-R rotation, P-S-R rotation had high amounts of βG. It may also be suggested that βG activity in P-S-R rotation gives a reflection of past biological activity.

This also is consistent with other earlier observations (Makoi and Ndakidemi, 2008; Badiane et al., 2001), which demonstrated that a significant fraction of βG activity in soil is associated with abiotic enzymes excretion into the soil solution or adsorption by clay and humic colloids.

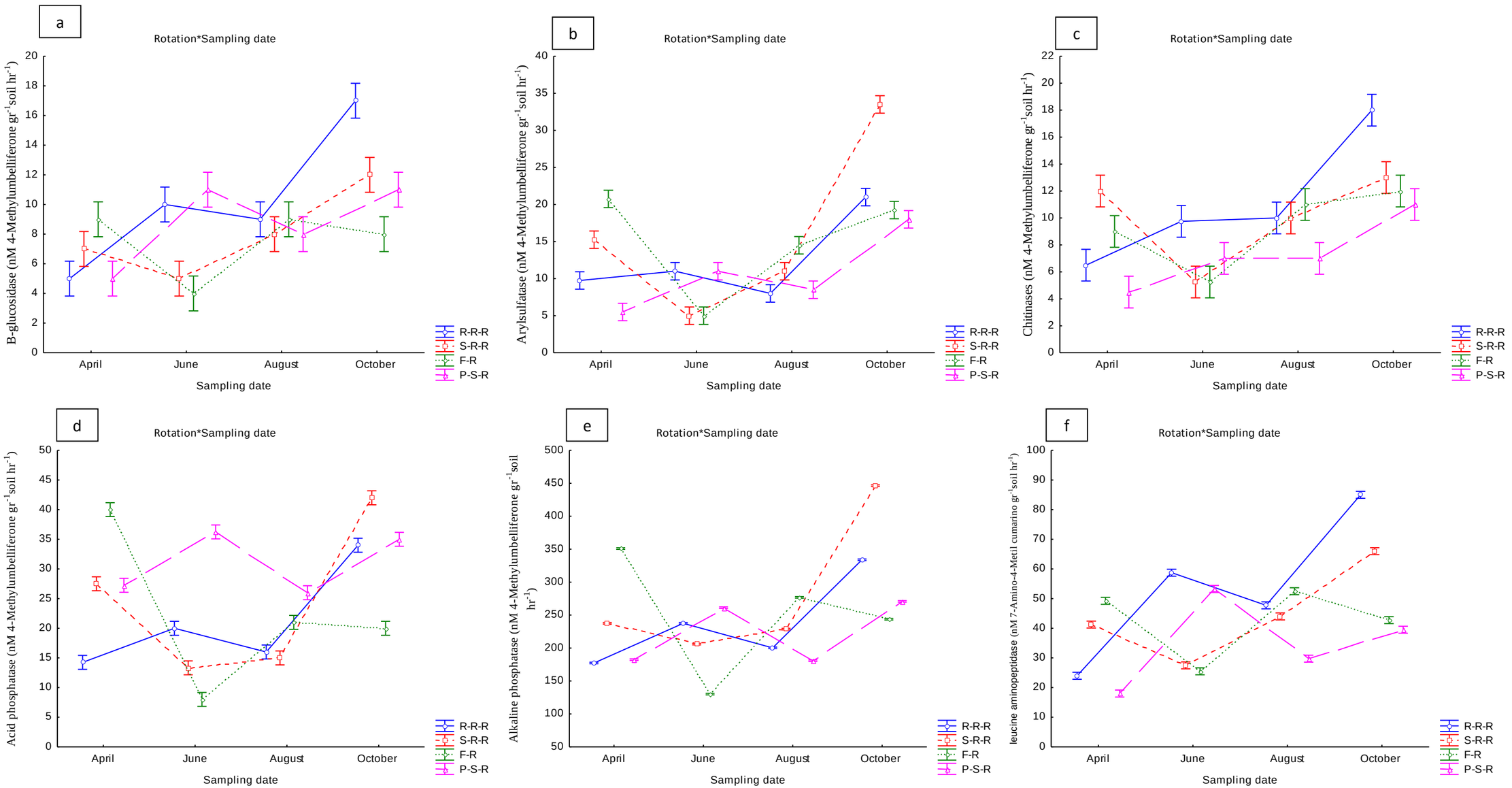


Fig. 17. Enzyme activities: a. B-glucosidase, b. Arylsulfatase, c. Chitinase, d. Acid phosphatase, e. Alkaline phosphatase, and F. leucine aminopeptidase in different rotation systems: Rice-Rice-Rice, Soya-Rice-Rice, Fallow-Rice and Pea-Soya-Rice at varying growth stages, April (after field preparation), June (after seedling), August (after tillering stage of rice in flooded condition) and October (after rice harvesting). The error bar at each data point represents the confidence intervals (0.95%).

3.5.1.2. *Arylsulfatase*

Arylsulfatase activity changed drastically during the growing season for each rotation. Compared to the drained-soil, all rotations in the early period of waterlogging resulted in decreased arylsulfatase activity, ranging from 14.90 % to 61 % depending on rotation system (Table 24). This was consistent with the results of other studies (Xia-chang and Qin, 2006; Tabatabai and Bremner, 1970) stating that drained-soil results in an increase in arylsulfatase activity, although, other research has shown that air-drying decreased arylsulfatase activity slightly (Bandick and Dick, 1999). The soil of S-R-R rotation had the highest arylsulfatase activity (average amount), whereas P-S-R rotation had a lower activity, of about half that of the S-R-R rotation. The highest arylsulfatase activity in S-R-R rotation in the month of October may be explained by high levels of organic carbon and high microbial respiration (Fig. 14b and 18d).

3.5.1.3. *Acid and alkaline phosphatases*

Acid phosphatase activity did not significantly change in S-R-R rotation with the increase in waterlogging time, whereas its activity in October was significantly higher ($P \leq 0.05$) than those in other months. Compared to the drained soil, early waterlogging (June) did not significantly decrease alkaline and acid phosphates activities in P-S-R rotation. The mean acid phosphatase activity in considered soils was found in the order (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1}): P-S-R (31.13) > S-R-R (24.46) > F-R (22.25) > R-R-R (21.06); thus its activity was higher under alternate cropping patterns of *gramineae* and legumes (i.e. P-S-R and S-R-R) compared to the monoculture rotations (i.e. R-R-R and F-R). Furthermore, enzyme activity in P-S-R and S-R-R rotations could be related to two contributions, one from the existing enzymes, and the other from new exudations of microbes and rice plants. Many studies have pointed out that the amount of acid phosphatase secreted by plant roots varies according to crop species,

varieties as well as management practices (Ndakidemi, 2006; Izaguirre-Mayoral and Carballo, 2002). In addition, Yadav and Tarafdar (2001) showed that legumes exude more phosphatase enzymes (72%) than cereals, which may probably be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals (Makoi and Ndakidemi, 2008).

Acid phosphatase activity was drastically lower in June than in October for all treatments, except P-S-R rotation; it could be due to the presence of slightly alkaline pH in early waterlogging compared to neutral pH in drained soil condition; the optimal pH range for acid phosphatase was reported to be about 3.5- 4.5 (Onthong et al., 2007).

Irrespective of rotation systems, the activity of acid phosphatase in October (drained-soil) was 150% that of the field-moist condition, whereas its activity in the drained-soil was only about 136 % of that of the waterlogged soil condition. This finding is in agreement with Xia-Chang and Qin (2006) who showed that acid and alkaline phosphatase activities in waterlogged soil were lower than that in air-dried soil (Table 20)**.**

A significantly positive correlation of fine silt particles and acid phosphatase activity was observed in this study. As might be expected, soil acid and alkaline phosphatase activities both negatively correlated with soil extractable phosphorus content (Sardans et al., 2008); acid phosphatase secretion is increased by plant roots due to P deficiency in the soil (Makoi and Ndakidemi, 2008), although there is no evidence of alkaline phosphatase activity with P deficiency (Šarapatka., et al, 2004).

The pattern for alkaline phosphatase activity at each rotation and measuring date was almost the same as that for acid phosphatase and showed significant differences ($P \leq 0.05$) (Fig. 17 d and e). It is ranging from 130 to 450 nM 4-Methylumbellifeone/gr.hr. The activity of

alkaline phosphatase was higher than that of acid phosphatase; it might be explained due to sufficiently high soil pH (i.e. neutral to moderately alkaline) throughout the growing season.

There was an inhibitory effect of waterlogging (except P-S-R rotation) for both alkaline and acid phosphatases in the month of June. In spite of being in waterlogged condition, the level of alkaline phosphatase activity in P-S-R rotation was almost high, the same as soil drained condition; this result is likely associated with high soil respiration values in this rotation system (Fig. 18d), since alkaline phosphatase activity derive only from microorganism and animals (Tabatabai, 1994; Alef et al., 1995).

3.5.1.4. *Chitinase*

Compared to field-moist soil (April), the R-R-R and P-S-R rotations at waterlogged soil condition (in the months of June and August) resulted in an increase in chitinase activity. Chitinase activity, in R-R-R and P-S-R rotations both did not change drastically with waterlogging time. The activities of chitinase in the waterlogged soils (in June) of S-R-R and F-R rotations were quite low (5.2), while increased markedly (up to 18) with soil drainage for all rotations. After rice harvesting (drained-soil), chitinase activities in the soils of the R-R-R and P-S-R rotations were 277% and 244% of those of the field-moist soil, respectively. The highest and lowest average level of chitinase activity (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1}) was found in R-R-R (11.07) and P-S-R (7.38) rotations, respectively. There was a significantly negative correlation between the chitinase activity and soil pH (Table 19).

Table 19-Pearson correlation coefficient between some physicochemical and biochemical soil properties

	Soil enzymes (nM-Methylumbelliferone gr^{-1} soil hr^{-1})						Soil properties							
	B-glucosidase	Arylsulfatase	Alkaline phosphatases	Acid phosphatases	Leucine aminopeptidase	Chitinase	pH	EC (dS m^{-1})	P (mg kg^{-1})	SOC (%)	Clay (%)	Coars Silt (%)	Fine Silt (%)	Sand (%)
B-glucosidase	1.00	0.65**	0.70**	0.65**	0.90**	0.83**	-0.54**	-0.05	-0.25	0.20	-0.04	-0.16	0.26	-0.08
Arylsulfatase	0.65**	1.00	0.92**	0.72**	0.67**	0.73**	-0.51**	0.04	-0.34*	0.55**	-0.22	-0.36*	0.18	0.12
Alkaline phosphatases	0.70**	0.92**	1.00	0.79**	0.76**	0.65**	-0.45**	0.22	-0.42**	0.63**	-0.18	-0.30*	0.28	0.01
Acid phosphatases	0.65**	0.72**	0.79**	1.00	0.51**	0.43**	-0.21	0.05	-0.45**	0.39**	0.29*	0.02	0.62**	-0.50**
Leucine aminopeptidase	0.90**	0.67**	0.76**	0.51**	1.00	0.83**	-0.56**	0.05	-0.16	0.28	-0.33*	-0.32*	0.05	0.22
Chitinase	0.83**	0.73**	0.65**	0.43**	0.83**	1.00	-0.49**	-0.21	-0.02	0.15	-0.33*	-0.32*	-0.09	0.31*
DNA	0.57**	0.74**	0.74**	0.52**	0.55**	0.51**	-0.74**	0.20	-0.59**	0.58**	-0.098	-0.182	0.314*	-0.085
pH	-0.54**	-0.51**	-0.45**	-0.21	-0.56**	-0.49**	1.00	-0.27	0.58**	-0.27	0.21	0.38**	-0.22	-0.10
EC (dS m^{-1})	-0.05	0.04	0.22	0.05	0.05	-0.21	-0.27	1.00	-0.48**	0.50**	-0.16	-0.30*	0.39**	-0.06
P (mg kg^{-1})	-0.25	-0.34*	-0.42**	-0.45**	-0.16	-0.02	0.58**	-0.48**	1.00	-0.43**	-0.13	0.02	-0.62**	0.42**
SOC (%)	0.20	0.55**	0.63**	0.39**	0.28	0.15	-0.27	0.50**	-0.43**	1.00	-0.18	-0.13	0.33*	-0.08
Clay (%)	-0.04	-0.22	-0.18	0.29*	-0.33*	-0.33*	0.21	-0.16	-0.13	-0.18	1.00	0.39**	0.44**	-0.81**
Coars Silt (%)	-0.16	-0.36*	-0.30*	0.02	-0.32*	-0.32*	0.38**	-0.30*	0.02	-0.13	0.39**	1.00	-0.04	-0.53**
Fine Silt (%)	0.26	0.18	0.28	0.62**	0.05	-0.09	-0.22	0.39**	-0.62**	0.33*	0.44**	-0.04	1.00	-0.77**
Sand (%)	-0.08	0.12	0.01	-0.50**	0.22	0.31*	-0.10	-0.06	0.42**	-0.08	-0.81**	-0.53**	-0.77**	1.00

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Table 20- multiple comparison of mean values of enzyme activities among four different rotation systems. Data in a column followed by the same letter are not significantly different at P = 0.05, two-way ANOVA with LSD test.

Enzyme	Crop rotation	Sampling date (growth stages)			
		April	June	August	October
B-glucosidase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	Rice-Rice-Rice	5c	10b	9a	17a
	Soya-Rice-Rice	7 b	5c	8b	12b
	Fallow-Rice	9a	4d	9a	8d
	Pea-Soya-Rice	5c	11a	8b	11c
Arylsulfatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	Rice-Rice-Rice	9.75c	11a	8d	21b
	Soya-Rice-Rice	15.25 b	5b	11b	33.5a
	Fallow-Rice	20.75a	5b	14.5a	19.25c
	Pea-Soya-Rice	5.5 d	11a	8.5c	18d
Chitinase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	Rice-Rice-Rice	6.5c	9.75a	10b	18a
	Soya-Rice-Rice	12a	5.25c	10b	13b
	Fallow-Rice	9b	5.25c	11a	12c
	Pea-Soya-Rice	4.5d	7b	7c	11d
Acid phosphatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	Rice-Rice-Rice	14.25c	20b	16c	34c
	Soya-Rice-Rice	27.5b	13.33c	15d	42a
	Fallow-Rice	40a	8d	21b	20d
	Pea-Soya-Rice	27.25b	36.25a	26a	35b
Alkaline phosphatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	Rice-Rice-Rice	177.25d	237.5b	200.3c	334b
	Soya-Rice-Rice	237b	206c	228.8b	446.5a
	Fallow-Rice	351a	130.3d	277a	243.8d
	Pea-Soya-Rice	182.5c	261.3a	180.8d	270.8c
leucine aminopeptidase (nM 7-Amino-4-Metil cumarino gr^{-1}soil hr^{-1})	Rice-Rice-Rice	24c	58.75a	47.75b	85a
	Soya-Rice-Rice	41.25b	27.50 c	44c	66b
	Fallow-Rice	49.25a	25.50d	52.5a	42.75c
	Pea-Soya-Rice	18d	53.25b	29.75d	39.5d

3.5.1.5. Leucine aminopeptidase

The leucine aminopeptidase activity varied as well as other enzyme activities depending on rotation and soil sampling time. The differences between treatments were significant ($P \leq 0.05$) at each measured date and rotation system during the entire growing period. Compared to the field-moist soil, waterlogging significantly increased leucine aminopeptidase activity in R-R-R and P-S-R rotations, although it decreased the activity of leucine aminopeptidase significantly in S-R-R rotation, while its activity increased with waterlogging time, and it was further increasing under drained-soil conditions. Leucine aminopeptidase activity had the following order: waterlogged soil (June)> drained-soil (October)> field-moist soil (April) in P-S-R rotation, while in R-R-R rotation it was drained-soil (October) > waterlogged (June) >field-moist soil (April).

The activity of leucine aminopeptidase in F-R rotation was drastically lower in the waterlogged soil (June) than in the drained-soil (October), while it was slightly higher in field-moist soil (April) compared to the activity in the drained-soil (October). Correlation analysis showed that leucine aminopeptidase activity was strongly affected by soil pH. R-R-R had the highest level of leucine aminopeptidase activity (53.87 nM 4-Methylumbelliferone gr^{-1}soil hr^{-1}).

3.5.2. Soil biological properties

Descriptive statistics of some soil biological properties, including Active C (mg kg^{-1}), PMN (mg g^{-1} $week^{-1}$), Basal soil respiration (µg C-Co_2 g^{-1} h^{-1}), Soil biological quality Index (QBS-ar), DNA (ng g^{-1}ds), and Act-c/SOC (%) are presented in table 21. In order to find out the effect of various rotation systems on investigated parameters throughout the growing season, the parameters were tested by two-way analysis of variance (ANOVA), and comparisons among treatment means were made using the LSD test calculated at $P < 0.05$ (Fig. 18).

Table 21- Descriptive statistics of some soil biological properties

Rotation	Soil biological properties						
		Active C (mg kg-^{1})	Act-c/SOC (%)	P.M.N (mg g^{-1} week^{-1})	BR (µg C-Co_2 g^{-1} h^{-1})	QBS-ar	DNA (ng g^{-1}ds)
R-R-R	Mean	682.04	5.26	6.52	0.19	50.92	15896.6
	Max[1]	805.13	7.20	16.11	0.28	66	21401
	Min[2]	605.66	3.12	0.15	0.07	15	7559
	CV[3] (%)	9.11	24.90	99.84	32.63	38.68	36.54
S-R-R	Mean	733.99	5.34	12.09	0.26	61.33	19675
	Max	854.51	8.29	45.77	0.43	80	37501
	Min	607.98	2.10	0.96	0.08	35	8699
	CV (%)	10.9	38.76	148.22	46.15	27.34	57.22
F-R	Mean	833.96	4.25	4.72	0.31	50.33	19900
	Max	1194.62	5.37	12.13	0.70	99	25901
	Min	480.58	3.15	0.91	0.17	20	9499
	CV (%)	40.42	19.05	81.77	45.16	59.74	33.30
P-S-R	Mean	736.42	4.92	17.49	0.25	44.92	18475
	Max	825.94	6.76	50.57	0.45	57	23101
	Min	638.92	3.36	1.90	0.14	20	10499
	CV (%)	10.66	22.15	109.09	39.2	22.39	28.24

[1] Max, maximum value.
[2] Min, minimum value.
[3] CV, coefficient of variance.

Active carbon ranged from 480 to 1194.62 mg kg^{-1}. Among the four rotations, F-R had the highest (1194.62 mg kg^{-1}) and the lowest (480 mg kg^{-1}) active carbon content and it was significantly correlated with porosity (r = - 0.67), AWC (0.49), OC (r = 0.64) and SmaI (r = 0.47). Our findings highlighted that the high amount of stable macroaggregates index (SmaI) in F-R rotation, during the months of April and August, may be affected by the elevated active carbon contents. However, the SmaI was not correlated to organic carbon. These results agree with the findings of other studies, which showed that the remained root of plants and their secretions in the period of growth may enhance the active soil organic carbon, which in turn increase soil aggregate stability (Li et al., 2012; Abiven et al., 2009; Six et al., 2004).

The soil respiration rates, CO_2 emission, were affected by variations in crop rotation, as well as in the sampling time. The highest basal respiration was found in the soil of F-R rotation during April and August, while S-R-R rotation had the highest Basal respiration in June and October; this result is likely associated with high active carbon values in these rotations (Fig. 19a). As might be expected, basal respiration was significantly correlated with active carbon (r = 0.50) and DNA (r = 0.38) at p value < 0.01.

According to the approach used to measure soil DNA, the obtained DNA results were assumed to be a simple reliable index of soil microbial biomass (Fornasier et al., 2014). The soil DNA values were positively ($p < 0.01$) affected by WFPS (r = 0.73), OC (r = 0.59), PMN (r = 0.53) and Basal respiration (r= 0.38) (Table. 3). However, two important biological parameters, namely, soil DNA and metabolic quotient were negatively correlated to soil TI stress (r = - 0.41, $p < 0.01$ and r = - 0.33, $p < 0.05$ respectively) (data not shown), suggesting that TI pollution has some effects on size, composition, and activity of microbial community. In addition, more

diversity of microarthropod groups was found in June and August sampling times when there was less TI pollution (see Fig. 15d and 19). Our results confirm the results of other earlier observations (Wahsha et al., 2012c; Ai-Jun et al., 2007; Yao et al., 2003), demonstrating the harmful effect of different heavy metals on soil microbial biomass and activity. However, the present findings, associated with metabolic quotient, appear to be different with respect to results obtained by Yao et al (2003), who described that increases in microbial metabolic quotients is indicative of stress (e.g heavy metal contamination).Since the correlation between soil TI content and DNA was better than that between TI and qCO_2, DNA could be considered as a most important biological indicator of soil quality and be closely associated with soil TI pollution.

PMN ranged from 0.15 (in R-R-R) to 50.57 (in P-S-R) mg g^{-1} $week^{-1}$. The PMN value plummeted in all crop sequence at waterlogged soil condition when compared with field-moist soil (April), while increased markedly with soil drainage for all rotations. Furthermore, as recorded earlier, the result of enzyme activities showed that leucine, aminopeptidase activity, which are involved in N cycling, increased under drained-soil condition as well; it is likely that greater root secretion and subsequent more nutrient concentration and most optimum air-filled porosity are responsible for these seasonal patterns.

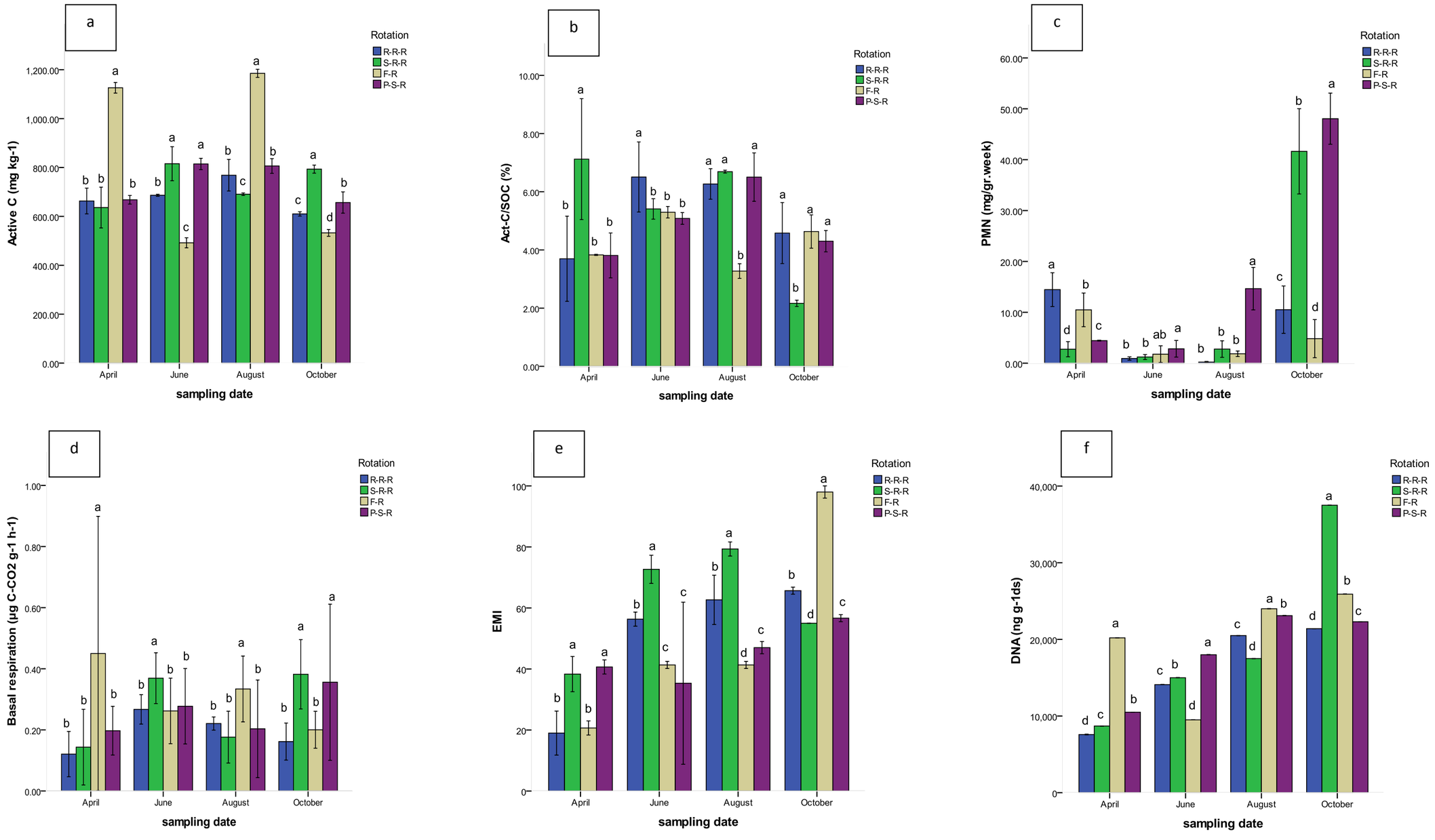


Fig. 18. Biological properties: a. Active C (mg kg-^{1}), b. Act-c/SOC (%), c.PMN (mg g^{-1} week^{-1}), d. Basal soil respiration (µg C-Co_2 g^{-1} h^{-1}), e. Soil biological quality Index (QBS-ar), and f. DNA (ng g^{-1}ds) of soils in different rotation systems: Rice-Rice-Rice, Soya-Rice-Rice, Fallow Rice and Pea-Soya-Rice. Error bars represent ± 2 S.D. from the mean soil chemical indicator value for each rotation. Different lowercase letters indicate significant differences among mean values of each rotation system over the rice growing season.

3.5.3. Soil biological quality Index (QBS-ar)

According to table 22, the QBS-ar values were found within a rather wide range (20 to 99). There were significant differences across rotation systems at each sampling period (Fig. 18e). The highest QBS-ar values occurred in S-R-R rotation in all sampling periods, except for the month of October, possibly because of the high active-C amount in this rotation. However, F-R rotation had the highest value of QBS-ar in drained soil condition. Nonetheless, It is noteworthy to point out that R-R-R resulted in the highest values of the QBS-ar after S-R-R rotation through the growing season.

Parisi et al. (2005) stated that, a stable ecosystem has QBS-ar values 100 to 200.The low QBS-ar values (i.e. < 100) and therefore having unstable ecosystem of study area may be explained through high soil contamination by Li and TI; it seems that heavy metal contamination of soils had significant impacts on soil microarthropod biodiversity

The classes of soil biological quality were determined based on the QBS-ar values for each system, as the approach explained in detail in chapter 2 (range 2 to 6). All cropping patterns demonstrated medium soil biological quality at the early period of waterlogging and drained soil condition, since they had a medium QBS-ar class equal to 3. However, the rotation systems present a low soil biological quality (i.e. class of soil quality = 2) in field-moist soil (April) in comparison to other sampling times. The lower soil biological qualities in field moist condition as compared to other soil moisture conditions are likely due to the lower water-holding capacity. The composition and abundance of total biological forms at the investigated systems is shown in Fig. 19. The eu-edaphic groups (Acarina and Collembola) were detected in most investigated rotations during the growing season. More diversity of microarthropod groups was found in June

and August sampling times. Figure 20 illustrates some microarthropod groups that were found under the study area (Italy).

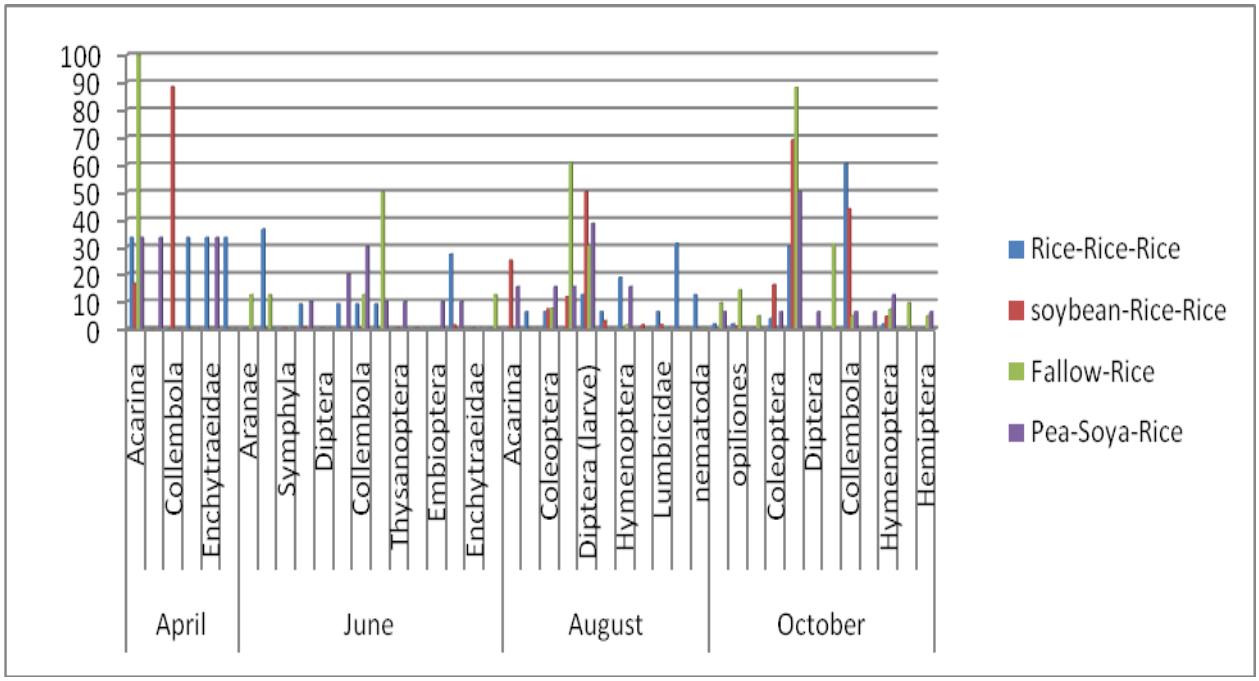


Fig. 19. The percentage frequency of soil fauna in different rotation systems over sampling dates.

Contrary to expectations, Correlation analysis showed that QBS-ar values were negatively correlated with AWC (-0.45) and Active-C (-0.37) (Table. 6).

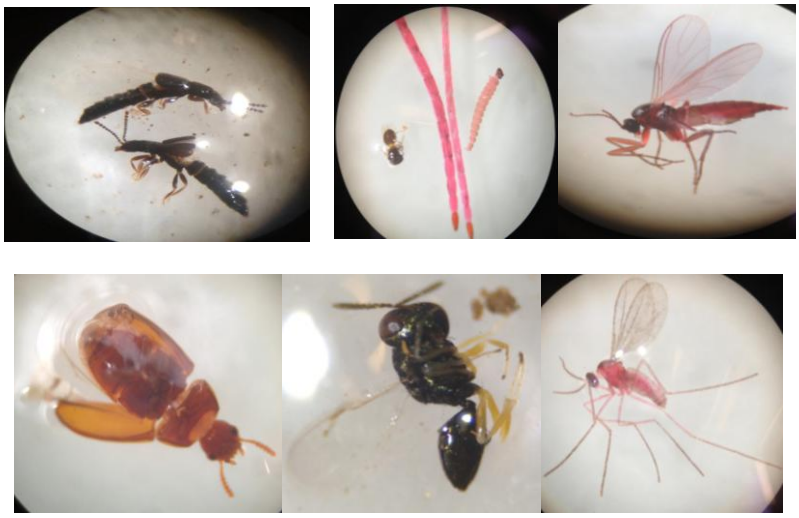


Fig. 20. Some of the microarthropod groups that were found under study area (Italy).

Table 22- Eco-morphological index, EMI, and QBS-ar values for investigated rotation systems over the rice-growing season.

Rotation	Biological forms	Number	Abundance	EMI	QBS-ar	Class of SQ
April						
R-R-R	Acarina	2	33.3	20	20	2
	Enchytraeidae	2	33.3	-		
	Gastropoda	2	33.3	-		
S-R-R	Acarina	1	16.6	20	40	3/2
	collembola	5	88.3	20		
F-R	Acarina	7	100	20	20	2
P-S-R	Acarina	2	33.3	20	40	2
	Diptera (larvae)	2	33.3	20		
	Enchytraeidae	2	33.3	-		
June						
R-R-R	Diplopoda	4	36.36	20	57	3
	Coleoptera	1	9.09	15		
	Collembola	1	9.09	10		
	Diptera (larvae)	1	9.09	10		
	Hemiptera	1	9.09	1		
	Hymenoptera	3	27.27	1		
S-R-R	Symphyla	1	0.19	20	74	3
	Diplopoda	1	0.19	20		
	Coleoptera	3	0.57	15		
	Collembola	2	0.38	10		
	Diptera	1	0.19	1		
	Hemiptera	1	0.19	5		
	Hymenoptera	7	1.33	1		
	Psocoptera	1	0.19	1		
	Thysanoptera	1	0.19	1		
	Enchytraeidae	1	0.19	-		
F-R	Aranae	1	12.5	5	41	3/2
	Diplopoda	1	12.5	20		
	Collembola	1	12.5	15		
	Hemiptera	4	50	1		
	Gastropoda	1	12.5	-		
P-S-R	Collembola	3	30	15	43	3/2
	Coleoptera	1	10	5		
	Diptera (larvae)	2	20	10		
	Hemiptera	1	10	1		
	Hymenoptera	1	10	1		
	Embioptera	1	10	10		
	Thysanoptera	1	10	1		
August						
R-R-R	Diplopoda	1	6.25	20	65	3
	Diptera (larve)	2	12.5	10		
	Lumbicidae	1	6.25	-		
	nematoda	2	12.5	-		
	Gastropoda	5	31.25	-		
	Coleoptera	1	6.25	10		
	Collembola	1	6.25	20		
	Hymenoptera	3	18.75	5		
S-R-R	Acarina	17	25	20	80	6
	Coleoptera	5	7.35	20		
	Lumbicidae	1	1.47	-		
	Enchytraeidae	1	1.47	-		
	Coleoptera (larve)	8	11.76	10		
	Diptera (larve)	34	50	10		
	Collembola	2	2.94	20		
F-R	Coleoptera	5	7.57	20	41	2
	Coleoptera (larve)	40	60.60	10		
	Diptera (larve)	20	30.30	10		
	Hymenoptera	1	1.5	1		
P-S-R	Acarina	2	15.38	20	47	2
	Coleoptera (Adult)	2	15.38	6		
	Coleoptera (larve)	2	15.38	10		
	Diptera (larve)	5	38.46	10		
	Hymenoptera	2	15.38	1		
October						
R-R-R	Acarina	1	1.88	20	66	3
	opiliones	1	1.88	10		
	Coleoptera	2	3.77	5		
	Coleoptera (larve)	16	30.18	10		
	Collembola	32	60.37	20		
	Hymenoptera	1	1.88	1		
S-R-R	opiliones	1	1.14	10	55	3
	Coleoptera	14	16.09	10		
	Coleoptera (larve)	60	68.96	10		
	Collembola	7	43.75	20		
	Hymenoptera	4	4.59	1		
F-R	Acarina	4	9.52	20	99	2/3
	Collembola	2	4.76	20		
	oplionida	6	14.28	10		
	Pauropoda	2	4.76	20		
	Gastropoda	4	9.52	-		
	Diptera (larve)	13	30.95	10		
	Coleoptera (larve)	37	88.09	10		
	Hymenoptera	3	7.14	5		
	Hemiptera	2	4.76	4		
P-S-R	Acarina	1	6.25	20	58	3
	Collembola	1	6.25	10		
	Diptera	1	6.25	1		
	hosphate n(Adult)	1	6.25	10		
	Coleoptera (larve)	8	50	10		
	Hemiptera	1	6.25	5		
	Hymenoptera	2	12.5	1		
	Thysanoptera	**1**	6.25	1		

3.6. Indicator selection based on Expert opinion

As described previously, three different soil quality indices were assessed based on 3 main steps (i.e. indicator selection, interpretation and integration indicator scores into a SQ index) (Fig 21). We used the suggested indicators from the SMAF method as an expert opinion so as to calculate Additive and weighted additive index. The indicators were SMAI (%), BD (g cm^{-3}), water filled pore space (%), EC (dS m^{-1}), PMN (mg g^{-1} $week^{-1}$), B-glucosidase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1}), pH, AWC (g g^{-1}), extractable phosphorous (ppm), and qCO2 (mg c-Co2/mg act C.Kg/h). In order to assess soil quality based on systematic soil quality index as an expert opinion, 25 MDS indicators were chosen from 62 physical, chemical and biological soil attributes according to the five soil functions of interest and the defined management goals for the rotation systems (Ferrarini et al., 2014; Sharma et al., 2005; Glover et al., 2000; Andrews et al., 2002a) (Tables 28 to 35).

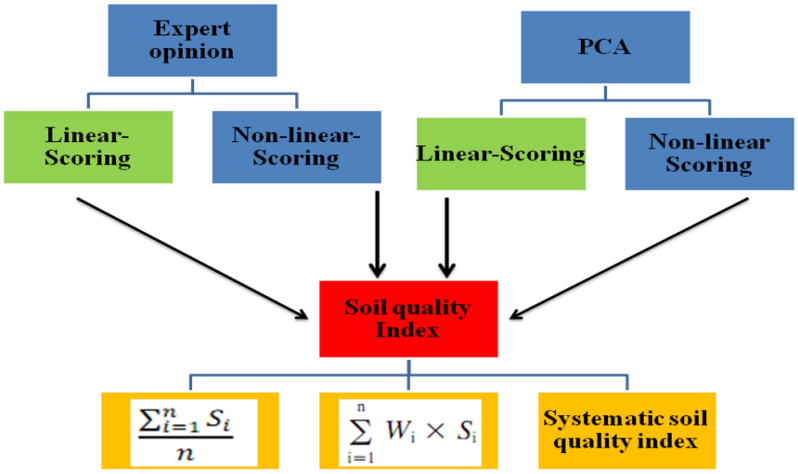


Fig. 21. Flow chart representing the three steps of soil quality indices development, which was applied in this study.

3.7. Indicator selection based on Principal Components analysis

The principal component analysis was performed for all different rotation systems over the rice growing season (April, June, August and October) which included sixty-two chemical, physical, and biological variables, using Statistica 7 software. Since the results of Two-way analysis of variance (ANOVA) revealed that stable aggregates index, stable macroaggregates index, and mineralization quotient have not significant differences among rotation systems in all considered sampling periods; therefore they were not considered for the PCA analysis.

Considering 62 soil chemical, physical and biological attributes previously mentioned, the PCA generated 62 PCs. According to Dunteman (1989), the principal components to keep were equal to seven since their eigenvalues were bigger than one. However, we noticed that the cut-off point in the scree plot (not shown) was at PC5, thus concerning this criterion the first five principal components with eigenvalues > 3 were kept to demonstrate the variability of the data set. A scree plot is a graphical display, in a descending order of magnitude, indicating the eigenvalues associated with each PC; in the scree test the PCs up to the elbow of the plot were considered in this study (Armenise et al., 2013). According to Kaiser criteria, when the eigenvalue is very close to one the diagnosis between keep/exclude of PCs may not be appropriate (Norman and Streiner, 2008).

These five principal components accounted for 85.96% of the whole data set (Table 23). The highly weighted variables under these PC_S were Al, As, Co, Cr, Fe, K, Li, Ni, Zn, AWC, CEC, Ct, B-glucosidase, Chitinase, pH, MWD s, WSA cw (%). The first principal component, which included, Al, As, Be, Co, Cr, Fe, K, Li, Ni, and Zn as highly weighted variables, explained 36.25 % of the variance. As described in detail in chapter two, a correlation matrix was subsequently run among the highly weighted variables under each principal component. We

identified the first component as the “trace elements component”; as it had the highest positive loading factors for the trace elements, Cobalt had the highest correlation sum than the other highly correlated variables in PC1. Moreover, since this component includes relatively high percentage (36.25%) of total variation of the entire data set, we also chose Al and Zn (with the lowest correlation sum), as representatives PC1 (Table 28). High concentration of Zn in the grains has been reported (Cakmak, 2009) to increase seed viability, seedling vigor and stand establishment under marginal conditions of rice cultivation. Instead, Al is known as one of the major constraints that limit nutrient uptake, growth and rice yields (Meriga et al., 2004). The second component, which accounted for 25.59% of the observed variance, showed the high loading factors for available water capacity, Cation exchange capacity and the cumulative value of mineralized carbon during the 28-day period of respiration (Ct). These variables were not correlated, so all the three variables were added to the MDS. We considered the second principal component as the “organic carbon component” since all soil attributes included in this PC were statistically correlated with organic carbon. The third principal component was determined as the “enzyme component”. It explained 11.45 % of the variance (Table 23). The two important variables for PC3, B-glucosidase and Chitinase, had a high correlation coefficient (> 0.80). We chose Chitinase to represent this PC since it had the highest factor loading (Table 23 and 24).

Only pH received a high factor loading under PC4, so the forth component was identified as the “acidity component”. This component explained 7.44 % of the variance. Under the last PC examined, water stable of aggregates obtained from capillary-wetted treatment (WSA wc) was correlated with the mean weight diameter obtained from slaked treatment (MWD s). WSA wc was included in the MDS because of its highest factor loading; The fifth principal component was assumed as “aggregate-size stability”, this component explained 5.23 % of variance (Table

	Principal components						
	PC1	**PC2**	**PC3**	**PC4**	**PC 5**	**PC 6**	
Eigen value[1]	**22.47**	**15.87**	**7.10**	**4.61**	**3.25**	2.07	
% Total variance	36.25	25.59	11.45	7.44	5.23	3.34	
Cumulative %	36.25	61.84	73.28	80.73	85.96	89.30	
Cumulative Eigenvalue	22.47	38.34	45.44	50.05	53.30	55.36	
	Eigen Vectors[2,3]						
Soil properties							**Communalities**
Moisture content (%)	-0.052	-0.199	0.080	0.039	0.089	-0.239	0.60
Porosity (%)	0.078	0.114	0.093	0.167	-0.214	0.008	0.42
BD(g cm^{-3})	0.067	0.123	0.109	0.192	-0.194	0.024	0.43
Water filled pore space (%)	-0.011	-0.154	0.186	0.178	-0.049	-0.251	0.51
Sand (%)	-0.157	0.157	0.058	-0.036	0.004	-0.007	0.88
Coarse Silt (%)	0.142	-0.030	-0.076	-0.068	-0.046	-0.192	0.50
Fine Silt (%)	0.072	-0.203	0.000	0.034	0.023	0.105	0.64
Clay (%)	0.165	-0.063	-0.081	0.107	-0.003	0.035	0.71
AWC (g g-1)	0.102	***-0.209***	-0.026	-0.031	0.072	0.000	0.80
MWD s (%)	-0.076	-0.100	0.127	-0.212	**-0.287**	0.020	0.47
MWD cw (%)	-0.134	-0.104	0.096	-0.154	-0.239	-0.051	0.66
WSA s (%)	-0.026	-0.090	0.162	-0.207	-0.258	0.183	0.35
WSA cw (%)	-0.051	-0.109	0.131	-0.212	***-0.312***	-0.110	0.42
GMDS (%)	-0.022	-0.089	0.196	-0.247	-0.273	0.107	0.43
GMDwc (%)	-0.018	-0.110	0.102	-0.152	-0.254	-0.286	0.29
PH	0.084	0.070	-0.116	***-0.324***	0.079	-0.017	0.48
C.E.C ($cmol^{+}$ kg^{-1})	0.041	***-0.230***	0.010	-0.107	0.093	0.089	0.74
P (mg kg^{-1})	0.031	0.194	0.055	-0.209	0.059	-0.081	0.60
CaCO3 (%)	-0.087	-0.181	-0.101	-0.040	0.010	-0.265	0.64
OC (%)	-0.090	-0.167	0.027	-0.010	0.205	-0.047	0.57
EC (dS m^{-1})	-0.098	-0.126	-0.172	0.022	0.082	0.103	0.55
Al (ppm)	***0.203***	-0.059	-0.021	0.040	-0.044	-0.014	0.97
As (ppm)	**0.202**	-0.042	-0.014	0.064	-0.004	-0.030	0.95
B (ppm)	0.117	-0.201	-0.057	-0.041	-0.023	-0.011	0.84
Ba(ppm)	0.178	-0.131	-0.042	0.002	-0.026	0.002	0.93
Be (ppm)	**0.209**	-0.003	0.020	0.018	-0.017	-0.060	0.98
Ca (ppm)	-0.098	-0.172	-0.176	0.101	-0.091	0.009	0.75
Cd (ppm)	0.186	-0.044	0.086	-0.114	0.003	0.100	0.86
Co (ppm)	***0.209***	-0.028	0.012	0.013	-0.011	0.011	0.99
Cr (ppm)	**0.196**	-0.083	-0.006	0.053	-0.022	-0.017	0.95
Cu (ppm)	0.185	-0.118	0.006	0.028	-0.004	0.011	0.94
Fe (ppm)	**0.209**	-0.012	0.024	0.026	-0.008	0.004	0.99
K (ppm)	**0.189**	-0.076	-0.088	0.076	-0.070	-0.026	0.92
Li (ppm)	**0.200**	-0.028	-0.009	0.023	-0.059	-0.001	0.91
Mg (ppm)	-0.153	-0.119	-0.152	0.061	-0.094	0.013	0.82
Mn (ppm)	0.180	-0.112	-0.071	-0.016	-0.087	-0.002	0.91
Na (ppm)	-0.015	-0.172	-0.217	0.053	-0.004	-0.069	0.59
Ni (ppm)	**0.193**	-0.090	-0.030	0.061	-0.034	-0.002	0.95
P (ppm)	-0.003	0.014	0.176	-0.318	0.189	0.149	0.34
Pb (ppm)	0.187	-0.087	0.060	-0.102	0.013	0.050	0.91
S (ppm)	-0.105	-0.197	0.001	-0.057	0.143	0.027	0.76
Sb (ppm)	0.131	0.123	0.106	-0.002	0.160	-0.138	0.64
Si (ppm)	-0.159	-0.074	-0.115	-0.056	0.159	-0.112	0.71
Sn (ppm)	0.179	-0.069	0.025	-0.072	0.085	-0.183	0.80
Sr (ppm)	-0.065	-0.198	-0.165	0.096	-0.040	0.010	0.73
Ti (ppm)	0.172	0.095	0.091	-0.112	0.032	0.039	0.84
Tl (ppm)	0.142	0.122	0.129	-0.102	0.139	0.067	0.74
V (ppm)	0.186	-0.116	-0.034	0.003	-0.022	0.006	0.95
Zn (ppm)	***0.195***	0.029	0.106	0.015	0.046	-0.010	0.91
PMN (mg/gr.week)	0.027	-0.121	0.127	0.165	0.025	0.237	0.32
Active C (mg kg^{-1})	-0.061	-0.169	-0.023	-0.213	0.175	-0.207	0.55
EMI	-0.061	0.046	0.039	0.258	-0.276	-0.156	0.29
B-glucosidase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	-0.002	-0.066	**0.291**	0.173	0.133	-0.029	0.48
Arylsulfatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	-0.076	-0.106	0.269	0.095	0.036	0.101	0.60
Chitinase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	-0.052	0.004	***0.313***	0.146	0.098	-0.100	0.52
Acid phosphatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	0.043	-0.172	0.215	0.020	0.056	0.139	0.62
Alkaline phosphatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	-0.079	-0.140	0.236	0.055	0.098	0.102	0.64
leucine aminopeptidase (nM 7-Amino-4-Metil cumarino gr^{-1}soil hr^{-1})	-0.065	-0.032	0.278	0.122	0.183	-0.022	0.48
Soil respiration (μg C-CO_2 g^{-1} h^{-1})	-0.081	-0.190	-0.044	-0.050	0.035	0.264	0.62
Ct[4]	-0.041	***-0.215***	0.061	-0.076	0.129	-0.040	0.66
Metabolic quotient	-0.050	-0.094	-0.099	0.112	-0.094	0.462	0.24
Soil DNA Content (ng g^{-1}_{ds})	-0.077	-0.153	0.161	0.181	-0.054	-0.117	0.61

Table 23 – Results of principal components analysis of sixty-two soil properties having statistical differences among the four different rotation systems.

[1] Boldface eigenvalues correspond to the PCs (> 3) examined for the index.
[2] Boldface factor loadings are assumed highly weighted.
[3] Bold-italic factor loadings correspond to the indicators included in the MDS.
[4] The cumulative value of soil respiration(mgr C- CO2/ kg.day) (during 1,4, 7, 14, 21, and 28 days of incubation)

Table 24- Correlation matrix for highly weighted variables under examined principal components.

Variables of PC1										
Correlation coefficients	Al	As	Be	Co	Cr	Fe	K	Li	Ni	Zn
Al	1.00	0.98	0.96	0.98	0.98	0.97	0.97	0.96	0.99	0.85
As	0.98	1.00	0.96	0.97	0.95	0.97	0.94	0.97	0.96	0.85
Be	0.96	0.96	1.00	0.99	0.92	0.99	0.89	0.96	0.91	0.93
Co	0.98	0.97	0.99	1.00	0.96	1.00	0.92	0.95	0.95	0.92
Cr	0.98	0.95	0.92	0.96	1.00	0.94	0.97	0.92	0.98	0.81
Fe	0.97	0.97	0.99	1.00	0.94	1.00	0.90	0.95	0.93	0.94
K	0.97	0.94	0.89	0.92	0.97	0.90	1.00	0.91	0.97	0.72
Li	0.96	0.97	0.96	0.95	0.92	0.95	0.91	1.00	0.91	0.84
Ni	0.99	0.96	0.91	0.95	0.98	0.93	0.97	0.91	1.00	0.79
Zn	0.85	0.85	0.93	0.92	0.81	0.94	0.72	0.84	0.79	1.00
Correlation sums	9.63	9.55	9.51	9.64	9.44	9.58	9.17	9.36	9.39	8.65
Variables of PC2										
Correlation coefficients	AWC	C.E.C	Ct							
AWC	1.00	0.88	0.62							
C.E.C	0.88	1.00	0.81							
Ct	0.62	0.81	1.00							
Correlation sums	2.50	2.69	2.43							
Variables of PC3	B-glucosidase	Chitinase								
B-glucosidase	1.00	0.82								
Chitinase	0.82	1.00								
Correlation sums	1.82	1.82								
Variables of PC5	MWD $_s$	WSA $_{cw}$								
MWD s	1.00	0.82								
WSA cw	0.82	1.00								
Correlation sums	1.82	1.82								

27). Finally, only nine soil properties were selected as the most dominant soil indicators among entire data set based on the PCA method.

3.8. Endpoint measures

In order to check the ability of both the EO and the PCA selected-MDSs to explain variability in end-point data representing sustainable management goals, we performed a stepwise multiple linear regression analysis (Table 25). Both the final nine PCA-MDSs and ten

EO-MDSs (a set of SMAF indicators), based on observed and non linear-scored values, were used as the independent variables and rice yield during the years 2010 to 2012 (55q/ha on average) and metal contamination (lithium and thallium), as the dependent variable.

The regression using 2010 yield (60q/ha) (as dependent variable) yielded a significant linear relationship (R^2 = 0.63) based on scored PCA-MDSs with AWC, Zn, CEC, Chitinase as the most important independent variables. Extractable phosphorous (mg kg^{-1}), B-glucosidase, water filled pore space (%), AWC, and PMN were the most significant variables based on observed EO-MDSs when applying the yield in 2011 as dependent variable (R^2 = 0.71). Instead, the regression of MDS variables with 2011 yield based on scored EO-MDSs had the lowest coefficient of determination (R^2 = 0.67).The highest coefficient of determination for 2012 yield (R^2 = 0.66) was found based on observed EO-MDSs, with Extractable phosphorous (mg kg^{-1}), B-glucosidase, water filled pore space (%), and SMAI (%) as the most important independent variables. The lithium regression resulted in an R^2 = 0.94 pertaining to observed PCA-MDSs, with Al, AWC, CEC and Co being the most significant variables. However, regression of thallium produced an R^2 = 0.68 related to scored PCA-MDSs in which Zn, CEC, Al and chitinase were highly significant. The most significant independent variables based on observed PCA-MDSs in the regression using TI as the dependent variable were Zn, CEC, and Co, resulting in R^2 = 0.58. Extractable phosphorous (mg kg^{-1}), B-glucosidase, and water filled pore space were the most dominant indicators of both 2011 and 2012 yields regardless rotation systems, suggesting that these indicators have a strong influence on rice production.

The results showed no clear dominance for PCA-MDSs (for both observed and transformed values) over the EO-MDSs or conversely (see EO versus PCA in Table 26). For instance, 2010 yield was poorly explained by the observed EO-MDSs (R^2 = 0.11), although a much better

explanation appeared when scored PCA-MDSs were used (R^2 = 0.63).These findings appear to be consistent with results reported by Andrews et al. (2002a) who found that both expert opinion- and multivariate statistic-selected MDS indicators performed equally well in explaining the variation in management goals.

Table 25- Multiple linear regression (Stepwise method) of observed and scored indicator values (as independent variables) against end-point variables (management goals as dependent variables).

Endpoint	Observed				Scored (non-linear)			
	EO[1]		PCA[2]		EO		PCA	
	Adjusted R^2	Most significant MDS_S	Adjusted R^2	Most significant MDS_S	Adjusted R^2	Most significant MDS_S	Adjusted R^2	Most significant MDS_S
Yield 2010 (q/ha)	0.11	AWC	0.15	Co	0.48	AWC, EC, PMN	**0.63**	AWC, Zn, CEC, Chitinase
Yield 2011(q/ha)	**0.71**	P, BG, WFPS, AWC, PMN	0.27	AWC	**0.67**	AWC, P, BG, qCO2	0.47	AWC, Chitinase
Yield 2012(q/ha)	0.66	P, BG, WFPS, SMAI	0.52	Zn, Al	0.61	P,EC, BG, WFPS, qCO2	0.46	CEC, Zn Ct, Al
Metal contamination (Li)	0.54	AWC, EC, BD, WFPS	**0.94**	Al AWC CEC Co	0.48	BD, EC, P, pH, AWC, qCO2	**0.68**	Zn, CEC Al Chitinase
Metal contamination (TI)	0.55	EC, WFPS, BG, AWC, P	0.46	Zn, AWC	0.25	EC	**0.58**	Zn, CEC, Co

[1] EO: MDS chosen by expert opinion (the SMAF date set)
[2] PCA: MDS determined by PCA of data from all crop rotation over the growing season

3.9. Indicator transformation (scoring)

3.9.1. Linear scores

After defining the minimum data set indicators either by expert opinion (EO-MDS) or by Principal Component Analysis (PCA-MDS), we scored each of the MDS variables based on both linear and non-linear scoring method.

Our results confirm those obtained by Andrews et al. (2002a), indicating that the linear scoring method results are highly affected by the range of each indicator since each observation is a proportion of the highest or lowest observation for "more is better" or "less is better" indicators, respectively. Moreover, if the high or low score is an outlier, all of the subsequent scores become incorrectly skewed. In some cases it seems that linear score was not reasonable. For instance, the linear scores of treatment means for active carbon ranged from 0.57 to 0.69, which were lower than was rational. Table 26 shows the comparison of rotations means and standard deviations of some measured indicator values with linear and non-linear transformed scores applied for the expert opinion and PCA-chosen minimum data sets.

3.9.2. Non-linear scores

Measured indicator values were transformed into non-linear values according to suggested mathematical algorithms in the SMAF and for the indicators where it has not been developed a SMAF scoring curve version, we used the general non linear scoring curve (NLSC) as described in detail in Chapter 2. Critical values, threshold and baseline values, based on literature, expert opinion, or specific data reference are summarized in Table 27.

In this research, threshold and baseline values for the most of chemical and biological indicators were determined according to the observed values, since there was not a well suited set of them for a paddy field in current literature. As stated in Andrews et al. (2002a), it appears

that the non linear scores demonstrate system function better than the linear scores. The non-linearly scored treatment means for extractable phosphorous (mg kg^{-1}), Active C (mg kg^{-1}) and SMAI (%) revealed that the observed values all fell within the optimum range for crop growth and environmental quality, while they were lower than the optimum range in the linearly scored treatment means (Table 26). Furthermore these non-linearly scored indicators have a much lower variation (%) between treatment means than their linearly scored counterpoints. Both the scoring methods seem to fulfill uniformly well for other indicators such as BD (g cm^{-3}), pH (- log H^{+}), OC (%) and DNA (ng g^{-1}_{ds}). The relative indicator scores using the linear and non-linear techniques for the EO-MDSs and PCA-MDSs in both the additive and the weighted additive indices are presented in Appendix 6 (comparing a and b, c and d, e and f, g and h).

3.10. Integration of Indicators into indices

Once the indicators were chosen by expert opinion and principal component analyses, they were transformed into linear and non-linear scores. In the third phase the scored indicators were subsequently combined into the examined soil quality indices (i.e. additive SQI, weighted additive SQI). The systematic SQI was studied using only linear and non-linear scored indicators chosen by EO-MDS selection technique. In order to evaluate rotation system effects on soil quality values, the soil quality indices were computed.

10.1. Additive soil quality index

The R-R-R rotation based on non-linear EO-MDSs presented a medium-high soil health status (0.74), whereas P-S-R rotation considering linear EO-MDSs proved to have the highest additive soil quality index value. However, regarding to PCA-MDSs either by linear or non-linear scoring method P-S-R rotation received significantly higher SQI values compared to the other cropping patterns.

Table 26- Comparison of treatments means over the growing season and standard deviations (in parentheses) of some measured indicator values with linear and non-linear transformed scores applied for the expert opinion and PCA-chosen minimum data sets.

Rotation	Porosity (%)	BD ($g\ cm^{-3}$)	AWC ($g\ g^{-1}$)	pH ($-\log H^{+}$)	C.E.C ($cmol^{+}\ kg^{-1}$)	P ($mg\ kg^{-1}$)	OC (%)	Chitinase ($nM\ 4\text{-}MB\ gr^{-1}soil\ hr^{-1}$)	DNA ($ng\ g^{-1}_{ds}$)	Active C ($mg\ kg^{-1}$)	SMAI (%)
	Measured indicator										
R-R-R	51.03 (8.36)	1.36 (0.21)	0.14 (0.01)	7.89 (0.35)	17.36 (1.43)	132.83 (33.8)	1.37 (0.34)	11.06 (4.51)	15897 (5809)	682.04 (62.16)	36.72 (9.38)
S-R-R	49.50 (4.25)	1.32 (0.12)	0.15 (0.02)	7.82 (0.40)	22.33 (7.90)	98.13 (46.5)	1.78 (1.16)	10.06 (3.23)	19675 (11258)	733.99 (80.24)	41.99 (7.61)
F-R	46.40 (7.58)	1.23 (0.19)	0.16 (0.03)	7.68 (0.38)	25.36 (11.59)	72.90 (9.99)	1.94 (1.12)	9.31 (2.83)	19900 (6627)	833.97 (337.09)	49.15 (13.78)
P-S-R	51.31 (2.02)	1.34 (0.09)	0.21 (0.01)	7.85 (0.39)	31.30 (2.21)	68.93 (13.7)	1.47 (0.25)	7.38 (2.58)	18475 (5218)	736.42 (78.57)	37.30 (11.16)
	Linear scoring										
R-R-R	0.88 (0.089)	0.75 (0.12)	0.62 (0.03)	0.76 (0.03)	0.42 (0.03)	0.35 (0.09)	0.36 (0.09)	0.58 (0.23)	0.42 (0.16)	0.57 (0.50)	0.49 (0.12)
S-R-R	0.92 (0.039)	0.76 (0.069)	0.64 (0.1)	0.76 (0.03)	0.54 (0.19)	0.56 (0.28)	0.47(0.31)	0.52 (0.16)	0.52 (0.16)	0.61 (0.67)	0.56 (0.10)
F-R	0.86 (0.096)	0.82 (0.12)	0.67 (0.14)	0.78 (0.03)	0.61 (0.28)	0.61 (0.084)	0.52 (0.30)	0.49 (0.14)	0.53 (0.16)	0.69 (0.28)	0.65(0.18)
P-S-R	0.97 (0.028)	0.74 (0.056)	0.90 (0.03)	0.76 (0.03)	0.75 (0.053)	0.66 (0.12)	0.39 (0.06)	0.38 (0.13)	0.49(0.27)	0.61 (0.66)	0.49 (0.14)
	Non-linear scoring										
R-R-R	0.84 (0.28)	0.78 (1.98)	0.57 (0.1)	0.75 (0.07)	0.21 (0.04)	0.98 (0.03)	0.37 (0.15)	0.56 (0.29)	0.40 (0.24)	0.90 (0.46)	0.83 (0.15)
S-R-R	0.97 (0.37)	0.87 (0.17)	0.62 (0.83)	0.76 (0.07)	0.42 (0.29)	1	0.46 (0.32)	0.56 (0.29)	0.46 (0.33)	0.93 (0.51)	0.88 (0.12)
F-R	0.75 (0.31)	0.97 (0.04)	0.67(0.09)	0.79 (0.07)	0.50 (0.39)	1	0.53 (0.33)	0.49(0.26)	0.57 (0.28)	0.97 (0.17)	0.98 (0.02)
P-S-R	0.99 (0.10)	0.72 (0.15)	0.76 (0.02)	0.76 (0.08)	0.79 (0.07)	1	0.42 (0.11)	0.30 (0.23)	0.51 (0.23)	0.93 (0.44)	0.85 (0.18)

Table 27– some soil quality indicators, scoring curve shape, thresholds, site-specific factors and references for the scoring curve used in this research.

Indicator	Scoring curve	Lower threshold	Upper threshold	Lower baseline	Optimum	Upper baseline	Slope	Site-spec. factors	References
	Physical properties								
Bulk density (g cm^{-3})	Less is better	0.67	2	1.31	-	-	-2.46	b c, d = f (texture, mineral)	Andrews et al. (2004)
Stable Aggregate Index (%)	More is better	43	115	80.05	-	-	0.027		Data set[1]
Stable Macroaggr. Index (%)	More is better	0	78.37	41.30	-	-	0.0359	d = f (OM, texture, Fe2O3)	Andrews et al. (2004)
Mean Weight Diameter (mm)	More is better	0	2.32	1.20	-	-	0.951	-	Data set
Porosity (%)	Optimum	20	62.11	40	49	60	0.128	-	Data set
Water-Filled Pore Space (%)	Optimum	15	105	30	-		0.0398	b = f (texture, iOM)	Andrews et al. (2004)
AWC (g/g)	More is better	0	0.22	0.16	-	-	-	a, b, c =f(Texture)	Andrews et al. (2004)
	Chemical properties								
pH (-log H^{+})	Optimum	3	8	4.20	6	7	-	-	Andrews et al. (2004) Lima, A.C.R. (2007)
C.E.C ($cmol^{+}$ kg^{-1})	More is better	0	47.02	24.12	-	-	0.048	-	Data set
Extractable P (mg kg^{-1})	Optimum								Andrews et al. (2004)
Organic carbon (%)	More is better	0	3	1.64	0.47	-	0.47	-	Data set
Electrical conductivity (dS m^{-1})	Optimum	0	-	0.5	2	3.48	-	b = f (method, crop, texture)	Andrews et al. (2004) Grattan et al. (2002)
Al (ppm)	Optimum	1152	-	18549	159000	-	2.31	-	Data set
Co (ppm)	Less is better	4.34	20	7.08	-	-	-0.16	-	Data set
Zn (ppm)	Optimum	14.5	-	54.28	155	-	0.061	-	Data set
	Biological properties								
EMI	More is better	0	100	51.74	-	-	0.019	-	Data set
PMN (mg/gr.week)	More is better	0	2.84	0.76	-	-	0.436	C = f (OM, texture, climate)	Andrews et al. (2004)
Active C (mg kg^{-1})	More is better	0	1194.62	420	-	-	0.002	-	Data set
B-glucosidase (nM 4-Methylumbelliferone gr^{1}soil hr^{-1})	More is better	0	16	8.62	-	-	0.12	-	Data set
Arylsulfatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	More is better	0	27	13.64	-	-	0.05	-	Data set
Chitinase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	More is better	0	17	9.48	-	-	0.117	-	Data set
Acid phosphatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	More is better	0	54.78	24.72	-	-	0.04	-	Data set
Alkaline phosphatase (nM 4-Methylumbelliferone gr^{-1}soil hr^{-1})	More is better	0	394.51	247.95	-	-	0.0047	-	Data set
leucine aminopeptidase (nM 7-Amino-4-Metil cumarino gr^{-1}soil hr^{-1})	More is better	0	96.47	44.121	-	-	0.0245	-	Data set
Soil respiration (µgr C-CO2g $^{-1}$. Hr^{-1})	More is better	0	0.44	0.25	-	-	3.42	-	Data set
Ct (mgr C- CO2 kg^{-1}.day^{-1})	More is better	20	167.68	74.34	-	-	0.012	-	Data set
Metabolic quotient (mg c-Co2/mg act C.Kg/h)	More is better	0	0.025	0.012	-	-	75.21	-	Data set
Soil DNA Content (ng g^{-1}_{ds})	More is better	0	31411.5	18459.4	-	-	0.0000514	-	Data set

[1] We found the thresholds based on cumulative normal distribution of data set, and scores were obtained from the standardized scoring functions (Wymore, 1993).

3.10.2 Weighted additive soil quality index

The F-R rotation had the highest SQI based on non linear EO-MDSs, while the results of linear scoring EO-MDSs showed that P-S-R has significantly higher SQI compared with the other rotation systems. However, the results of index values on the basis of PCA for both linear and non-linear scores demonstrated that P-S-R resulted in the highest overall soil quality, followed by R-R-R and F-R systems.

3.10.3. Systematic Soil quality index

The systematic soil quality index was assessed considering the approach proposed for assessing grain production systems by Karlen and Stott (1994), in terms of five soil functions: 1) physical stability and structural support; 2) water relations; 3) nutrient cycling; 4) filtering and buffering; and 5) biodiversity and habitat. All five soil functions were considered to be equally important for the management goals (i.e. rice productivity and environmental protection) and were assigned weights of 0.20. We therefore developed a framework pertaining twenty- five soil quality indicators to the five soil functions. Almost all soil quality indicators suggested by Andrews et al. (2004) (i.e.soil management assessment framework), were included in the list of minimum data sets (i.e. in the five soil functions). However, our list also comprised some other properties such as, toxic elements (As, Li, TI), six important enzymes involved in C, N, P, and S cycling, active carbon and CEC. Each indicator has been dedicated to different levels associated with particular soil function. Within each indicator level, numerical weights were assigned to soil quality indicators based on their assumed importance to the specific soil function under consideration.

Briefly, the systematic soil quality index was obtained through multiplying each soil function score by its weight and summing up these values in order to understand each property's total influence within this particular soil quality index.

Macroaggregate stability, mean weighted diameter, organic carbon, bulk density and soil respiration were assumed as level one indicators so as to evaluate physical stability and structural support. Facilitate water movement and availability, stable Aggregates index, bulk density, and electrical conductivity were considered as level one indicators for water relation function. Facilitate water movement and availability was further broken down to the second level indicators of water-filled pore space, porosity, and organic carbon. C cycle, N cycle, P cycle, pH, C.E.C, water-filled pore space, and arylsulfatase (S cycle) were deemed as level one indicators of the soil's ability to sustain plant growth. However, C, N, and P cycle were broken to the second level indicators. Toxicity, biological activities and biodiversity, DNA content, pH, and SOC were used as main level one indicators of the soil's ability to filter and buffer high concentration of nutrients and pollutants. Instead, enzymes, DNA content, QBS-ar and P.M.N were supposed to be principal indicators level one of the soil's ability to support plant and animal life.

The results of systematic soil quality index based on linear and non-linear scoring for each rotation system throughout the growing season are presented in Tables 28 to 35. Combination of twenty-five soil attributes based on non-linear scores into the Systematic Soil quality index resulted in S-R-R cropping pattern getting a significantly higher score (0.59) than other rotation systems due to the higher performance of soil functions (i.e. simplify water transfer and absorption, sustain plant growth, filter and buffer high concentration of nutrients and pollutants, support plant and animal life) found in this rotation. The greater values of soil enzyme activity and soil biological quality Index (QBS-ar) of soil under S-R-R rotation were responsible for these variations. However, considering linear-scores similar soil quality index values were obtained for both S-R-R and F-R (0.54), which were higher than the other cropping patterns followed, by P-S-R and R-R-R rotation systems.

Table 28 – Soil quality score card for R-R-R rotation system using non-linear transformed data of expert opinion-chosen MDS.

SOIL FUNCTION	WEIGHT	SCORE	VALUE	INDICATOR Level 1	WEIGHT	SCORE	VALUE	INDICATOR Level 2	WEIGHT	SCORE	VALUE
Physical Stability and Structural Support	**0.2**	**0.60**	**0.12**	Macroaggregate Stability	0.4	0.84	0.34				
				Mean Weighted Diameter	0.1	0.37	0.04				
				Organic carbon	0.3	0.38	0.11				
				Bulk density	0.1	0.78	0.08				
				Respiration	0.1	0.33	0.03				
Water relations	**0.2**	**0.69**	**0.13**	**Facilitate water movement and availability**	0.4	0.59	0.23	Water-filled pore space	0.3	0.78	0.23
								Porosity	0.3	0.83	0.24
								Organic carbon	0.3	0.38	0.11
				Stable Aggregates Index	0.2	0.50	0.10				
				Bulk density	0.2	0.78	0.16				
				Electrical conductivity	0.2	1	0.20				
Nutrient Cycling	**0.2**	**0.54**	**0.1**	**C cycle**	0.3	0.56	0.16	SOC	0.3	0.38	0.11
								Active Carbon	0.3	0.89	0.27
								ß-glucosidase	0.2	0.58	0.12
								Respiration	0.2	0.33	0.07
				N cycle				P.M.N.	0.4	0.09	0.036
					0.2	0.39	0.078	Leucine aminopeptidase	0.4	0.62	0.24
								Chitinase	0.2	0.57	0.11
				P cycle	0.2	0.76	0.15	P available	0.6	0.98	0.58
								alkaline phosphatases	0.4	0.45	0.18
				pH	0.075	0.76	0.057				
				C.E.C.	0.075	0.22	0.016				
				Water-filled pore space	0.075	0.78	0.058				
				Arylsulfatase (S cycle)	0.075	0.44	0.033				
Filtering and Buffering	**0.2**	**0.43**	**0.086**	**Toxicity**	0.3	0.57	0.17	Arsenic	0.2	1	0.20
								Lithium	0.4	0.64	0.26
								Thallium	0.4	0.28	0.11
				Biological activities and biodiversity	0.3	0.28	0.084	P.M.N	0.3	0.09	0.03
								Respiration	0.3	0.33	0.10
								QBS-ar	0.3	0.52	0.16
				DNA content	0.1	0.41	0.04				
				pH	0.1	0.76	0.07				
				SOC	0.2	0.38	0.07				
Biodiversity and Habitat	**0.2**	**0.38**	**0.076**	**Enzymes**	0.25	0.48	0.12	B-glucosidase	0.16	0.58	0.09
								alkaline hosphate	0.16	0.45	0.07
								acid phosphatase	0.16	0.37	0.06
								leucine aminopept	0.16	0.62	0.1
								arylsulfatase	0.16	0.44	0.07
								chitinase	0.16	0.57	0.09
				DNA content	0.25	0.41	0.10				
				QBS-ar	0.25	0.52	0.13				
				P.M.N	0.25	0.09	0.02				
Environmental Protection and productivity		**Overall SQI**		**0.51**							

Table 29 –Soil quality score card for S-R-R rotation system using non linear transformed data of expert opinion-chosen MDS.

SOIL FUNCTION	WEIGHT	SCORE	VALUE	INDICATOR Level 1	WEIGHT	SCORE	VALUE	INDICATOR Level 2	WEIGHT	SCORE	VALUE
Physical Stability and Structural Support	**0.2**	**0.68**	**0.13**	Macroaggregate Stability	0.4	0.89	0.36				
				Mean Weighted Diameter	0.1	0.41	0.04				
				Organic carbon	0.3	0.46	0.14				
				Bulk density	0.1	0.87	0.09				
				Respiration	0.1	0.54	0.05				
Water relations	**0.2**	**0.7**	**0.14**	**Facilitate water movement and availability**	0.4	0.61	0.24	Water-filled pore space	0.3	0.63	0.19
								Porosity	0.3	0.97	0.29
								Organic carbon	0.3	0.46	0.14
				Stable Aggregates Index	0.2	0.57	0.11				
				Bulk density	0.2	0.87	0.17				
				Electrical conductivity	0.2	0.85	0.17				
Nutrient Cycling	**0.2**	**0.6**	**0.12**	**C cycle**	0.3	0.61	0.18	SOC	0.3	0.46	0.14
								Active Carbon	0.3	0.92	0.28
								ß-glucosidase	0.2	0.43	0.09
								Respiration	0.2	0.54	0.11
				N cycle	0.2	0.42	0.08	P.M.N.	0.4	0.26	0.1
								Leucine aminopeptidase	0.4	0.50	0.2
								Chitinase	0.2	0.57	0.11
				P cycle	0.2	0.82	0.16	P available	0.6	1	0.6
								alkaline phosphatases	0.4	0.54	0.21
				pH	0.075	0.77	0.06				
				C.E.C.	0.075	0.43	0.03				
				Water-filled pore space	0.075	0.63	0.05				
				Arylsulfatase (S cycle)	0.075	0.52	0.04				
Filtering and Buffering	**0.2**	**0.58**	**0.11**	**Toxicity**	0.3	0.75	0.22	Arsenic	0.2	1	0.2
								Lithium	0.4	0.68	0.27
								Thallium	0.4	0.70	0.28
				Biological activities and biodiversity	0.3	0.43	0.12	P.M.N	0.3	0.26	0.08
								Respiration	0.3	0.54	0.16
								QBS-ar	0.3	0.63	0.19
				DNA content	0.1	0.47	0.04				
				pH	0.1	0.77	0.07				
				SOC	0.2	0.46	0.92				
Biodiversity and Habitat	**0.2**	**0.46**	**0.092**	**Enzymes**	0.25	0.48	0.12	B-glucosidase	0.16	0.43	0.07
								alkaline hosphate	0.16	0.54	0.09
								acid phosphatase	0.16	0.46	0.07
								leucine aminopept	0.16	0.50	0.08
								arylsulfatase	0.16	0.52	0.08
								chitinase	0.16	0.57	0.09
				DNA content	0.25	0.47	0.12				
				QBS-ar	0.25	0.63	0.16				
				P.M.N	0.25	0.26	0.07				
Environmental Protection and productivity		**Overall SQI**		**0.59**							

Table 30 – Soil quality score card for F-R rotation system using non linear transformed data of expert opinion-chosen MDS.

SOIL FUNCTION	WEIGHT	SCORE	VALUE	INDICATOR Level 1	WEIGHT	SCORE	VALUE	INDICATOR Level 2	WEIGHT	SCORE	VALUE
Physical Stability and Structural Support	**0.2**	**0.78**	**0.15**	Macroaggregate Stability	0.4	0.99	0.4				
				Mean Weighted Diameter	0.1	0.66	0.07				
				Organic carbon	0.3	0.53	0.16				
				Bulk density	0.1	0.97	0.1				
				Respiration	0.1	0.61	0.06				
Water relations	**0.2**	**0.66**	**0.13**	**Facilitate water movement and availability**	0.4	0.51	0.20	Water-filled pore space	0.3	0.42	0.13
								Porosity	0.3	0.74	0.22
								Organic carbon	0.3	0.53	0.16
				Stable Aggregates Index	0.2	0.55	0.11				
				Bulk density	0.2	0.97	0.19				
				Electrical conductivity	0.2	0.78	0.15				
Nutrient Cycling	**0.2**	**0.58**	**0.11**	**C cycle**	0.3	0.64	0.19	SOC	0.3	0.53	0.16
								Active Carbon	0.3	0.92	0.28
								ß-glucosidase	0.2	0.40	0.08
								Respiration	0.2	0.61	0.12
				N cycle	0.2	0.31	0.062	P.M.N.	0.4	0.04	0.02
								Leucine aminopeptidase	0.4	0.48	0.19
								Chitinase	0.2	0.50	0.1
				P cycle	0.2	0.81	0.16	P available	0.6	1	0.6
								alkaline phosphatases	0.4	0.52	0.20
				pH	0.075	0.79	0.06				
				C.E.C.	0.075	0.51	0.04				
				Water-filled pore space	0.075	0.42	0.03				
				Arylsulfatase (S cycle)	0.075	0.56	0.04				
Filtering and Buffering	**0.2**	**0.59**	**0.11**	**Toxicity**	0.3	0.87	0.26	Arsenic	0.2	1	0.2
								Lithium	0.4	0.84	0.34
								Thallium	0.4	0.84	0.34
				Biological activities and biodiversity	0.3	0.32	0.09	P.M.N	0.3	0.04	0.01
								Respiration	0.3	0.61	0.18
								QBS-ar	0.3	0.41	0.12
				DNA content	0.1	0.57	0.06				
				pH	0.1	0.79	0.08				
				SOC	0.2	0.53	0.11				
Biodiversity and Habitat	**0.2**	**0.37**	**0.074**	**Enzymes**	0.25	0.46	0.11	B-glucosidase	0.16	0.40	0.06
								alkaline hosphate	0.16	0.52	0.08
								acid phosphatase	0.16	0.41	0.07
								leucine aminopept	0.16	0.48	0.08
								arylsulfatase	0.16	0.56	0.09
								chitinase	0.16	0.50	0.08
				DNA content	0.25	0.57	0.14				
				QBS-ar	0.25	0.41	0.10				
				P.M.N	0.25	0.04	0.01				
Environmental Protection and productivity		**Overall SQI**		**0.57**							

Table 31 – Soil quality score card for P-S-R rotation system using non linear transformed data of expert opinion-chosen MDS.

SOIL FUNCTION	WEIGHT	SCORE	VALUE	INDICATOR Level 1	WEIGHT	SCORE	VALUE	INDICATOR Level 2	WEIGHT	SCORE	VALUE
Physical Stability and Structural Support	**0.2**	**0.63**	**0.12**	Macroaggregate Stability	0.4	0.85	0.34				
				Mean Weighted Diameter	0.1	0.38	0.04				
				Organic carbon	0.3	0.42	0.13				
				Bulk density	0.1	0.72	0.07				
				Respiration	0.1	0.50	0.05				
Water relations	**0.2**	**0.44**	**0.088**	**Facilitate water movement and availability**	0.4	0.57	0.22	Water-filled pore space	0.3	0.50	0.15
								Porosity	0.3	0.99	0.30
								Organic carbon	0.3	0.42	0.13
				Stable Aggregates Index	0.2	0.48	0.10				
				Bulk density	0.2	0.72	0.14				
				Electrical conductivity	0.2	1	0.20				
Nutrient Cycling	**0.2**	**0.57**	**0.114**	**C cycle**	0.3	0.61	0.18	SOC	0.3	0.42	0.13
								Active Carbon	0.3	0.92	0.28
								ß-glucosidase	0.2	0.52	0.10
								Respiration	0.2	0.50	0.10
				N cycle	0.2	0.34	0.06	P.M.N.	0.4	0.35	0.14
								Leucine aminopeptidase	0.4	0.34	0.14
								Chitinase	0.2	0.31	0.06
				P cycle	0.2	0.76	0.15	P available	0.6	1	0.6
								alkaline phosphatases	0.4	0.40	0.16
				pH	0.075	0.76	0.06				
				C.E.C.	0.075	0.79	0.06				
				Water-filled pore space	0.075	0.50	0.04				
				Arylsulfatase (S cycle)	0.075	0.37	0.03				
Filtering and Buffering	**0.2**	**0.42**	**0.084**	**Toxicity**	0.3	0.36	0.10	Arsenic	0.2	1	0.2
								Lithium	0.4	0.04	0.02
								Thallium	0.4	0.37	0.15
				Biological activities and biodiversity	0.3	0.37	0.11	P.M.N	0.3	0.35	0.11
								Respiration	0.3	0.50	0.15
								QBS-ar	0.3	0.38	0.11
				DNA content	0.1	0.51	0.05				
				pH	0.1	0.76	0.08				
				SOC	0.2	0.42	0.08				
Biodiversity and Habitat	**0.2**	**0.41**	**0.082**	**Enzymes**	0.25	0.42	0.10	B-glucosidase	0.16	0.52	0.08
								alkaline hosphate	0.16	0.40	0.06
								acid phosphatase	0.16	0.71	0.11
								leucine aminopept	0.16	0.34	0.05
								arylsulfatase	0.16	0.37	0.06
								chitinase	0.16	0.31	0.05
				DNA content	0.25	0.51	0.13				
				QBS-ar	0.25	0.38	0.10				
				P.M.N	0.25	0.35	0.09				
Environmental Protection and productivity		**Overall SQI**		**0.48**							

Table 32 – Soil quality score card for R-R-R rotation system using linear transformed data of expert opinion-chosen MDS.

SOIL FUNCTION	WEIGHT	SCORE	VALUE	INDICATOR Level 1	WEIGHT	SCORE	VALUE	INDICATOR Level 2	WEIGHT	SCORE	VALUE
Physical Stability and Structural Support	**0.2**	**0.46**	**0.092**	Macroaggregate Stability	0.4	0.50	0.2				
				Mean Weighted Diameter	0.1	0.43	0.043				
				Organic carbon	0.3	0.37	0.11				
				Bulk density	0.1	0.75	0.08				
				Respiration	0.1	0.27	0.03				
Water relations	**0.2**	**0.69**	**0.13**	**Facilitate water movement and availability**	0.4	0.52	0.20	Water-filled pore space	0.3	0.48	0.14
								Porosity	0.3	0.88	0.26
								Organic carbon	0.3	0.37	0.11
				Stable Aggregates Index	0.2	0.78	0.16				
				Bulk density	0.2	0.75	0.15				
				Electrical conductivity	0.2	0.91	0.18				
Nutrient Cycling	**0.2**	**0.44**	**0.088**	**C cycle**	0.3	0.45	0.13	SOC	0.3	0.37	0.11
								Active Carbon	0.3	0.57	0.17
								ß-glucosidase	0.2	0.57	0.11
								Respiration	0.2	0.27	0.05
				N cycle	0.2	0.42	0.084	P.M.N.	0.4	0.13	0.052
								Leucine aminopeptidase	0.4	0.63	0.25
								Chitinase	0.2	0.58	0.11
				P cycle	0.2	0.42	0.084	P available	0.6	0.35	0.21
								alkaline phosphatases	0.4	0.53	0.21
				pH	0.075	0.76	0.06				
				C.E.C.	0.075	0.42	0.03				
				Water-filled pore space	0.075	0.48	0.04				
				Arylsulfatase (S cycle)	0.075	0.36	0.03				
Filtering and Buffering	**0.2**	**0.45**	**0.09**	**Toxicity**	0.3	0.62	0.18	Arsenic	0.2	0.66	0.13
								Lithium	0.4	0.80	0.32
								Thallium	0.4	0.41	0.16
				Biological activities and biodiversity	0.3	0.27	0.081	P.M.N	0.3	0.13	0.04
								Respiration	0.3	0.27	0.08
								QBS-ar	0.3	0.51	0.15
				DNA content	0.1	0.43	0.04				
				pH	0.1	0.76	0.08				
				SOC	0.2	0.37	0.07				
Biodiversity and Habitat	**0.2**	**0.39**	**0.078**	**Enzymes**	0.25	0.51	0.12	B-glucosidase	0.16	0.57	0.09
								alkaline hosphate	0.16	0.53	0.08
								acid phosphatase	0.16	0.49	0.08
								leucine aminopept	0.16	0.63	0.1
								arylsulfatase	0.16	0.36	0.06
								chitinase	0.16	0.58	0.09
				DNA content	0.25	0.43	0.11				
				QBS-ar	0.25	0.51	0.13				
				P.M.N	0.25	0.13	0.03				
Environmental Protection and productivity		**Overall SQI**		**0.47**							

Table 33 – Soil quality score card for S-R-R rotation system using linear transformed data of expert opinion-chosen MDS.

SOIL FUNCTION	WEIGHT	SCORE	VALUE	INDICATOR Level 1	WEIGHT	SCORE	VALUE	INDICATOR Level 2	WEIGHT	SCORE	VALUE
Physical Stability and Structural Support	**0.2**	**0.51**	**0.10**	Macroaggregate Stability	0.4	0.52	0.21				
				Mean Weighted Diameter	0.1	0.45	0.05				
				Organic carbon	0.3	0.48	0.14				
				Bulk density	0.1	0.76	0.08				
				Respiration	0.1	0.38	0.04				
Water relations	**0.2**	**0.73**	**0.14**	**Facilitate water movement and availability**	0.4	0.58	0.23	Water-filled pore space	0.3	0.53	0.16
								Porosity	0.3	0.92	0.28
								Organic carbon	0.3	0.48	0.14
				Stable Aggregates Index	0.2	0.83	0.17				
				Bulk density	0.2	0.76	0.15				
				Electrical conductivity	0.2	0.89	0.18				
Nutrient Cycling	**0.2**	**0.50**	**0.1**	**C cycle**	0.3	0.49	0.14	SOC	0.3	0.48	0.14
								Active Carbon	0.3	0.61	0.18
								ß-glucosidase	0.2	0.44	0.09
								Respiration	0.2	0.38	0.08
				N cycle	0.2	0.41	0.082	P.M.N.	0.4	0.24	0.1
								Leucine aminopeptidase	0.4	0.52	0.21
								Chitinase	0.2	0.53	0.11
				P cycle	0.2	0.59	0.11	P available	0.6	0.56	0.34
								alkaline phosphatases	0.4	0.63	0.25
				pH	0.075	0.77	0.058				
				C.E.C.	0.075	0.54	0.041				
				Water-filled pore space	0.075	0.53	0.04				
				Arylsulfatase (S cycle)	0.075	0.47	0.035				
Filtering and Buffering	**0.2**	**0.55**	**0.11**	**Toxicity**	0.3	0.75	0.22	Arsenic	0.2	0.77	0.15
								Lithium	0.4	0.83	0.33
								Thallium	0.4	0.66	0.26
				Biological activities and biodiversity	0.3	0.37	0.11	P.M.N	0.3	0.24	0.07
								Respiration	0.3	0.38	0.11
								QBS-ar	0.3	0.62	0.19
				DNA content	0.1	0.45	0.05				
				pH	0.1	0.77	0.08				
				SOC	0.2	0.48	0.10				
Biodiversity and Habitat	**0.2**	**0.45**	**0.09**	**Enzymes**	0.25	0.51	0.12	B-glucosidase	0.16	0.44	0.07
								alkaline hosphate	0.16	0.63	0.1
									0.16	0.57	0.09
								acid phosphatase	0.16	0.52	0.08
								leucine aminopept	0.16	0.47	0.08
								arylsulfatase	0.16	0.53	0.08
								chitinase			
				DNA content	0.25	0.45	0.11				
				QBS-ar	0.25	0.62	0.16				
				P.M.N	0.25	0.24	0.06				
Environmental Protection and productivity		**Overall SQI**		**0.54**							

Table 34 – Soil quality score card for F-R rotation system using linear transformed data of expert opinion-chosen MDS.

SOIL FUNCTION	WEIGHT	SCORE	VALUE	INDICATOR Level 1	WEIGHT	SCORE	VALUE	INDICATOR Level 2	WEIGHT	SCORE	VALUE
Physical Stability and Structural Support	**0.2**	**0.57**	**0.11**	Macroaggregate Stability	0.4	0.54	0.22				
				Mean Weighted Diameter	0.1	0.66	0.07				
				Organic carbon	0.3	0.52	0.16				
				Bulk density	0.1	0.83	0.08				
				Respiration	0.1	0.44	0.04				
Water relations	**0.2**	**0.75**	**0.15**	**Facilitate water movement and availability**	0.4	0.62	0.24	Water-filled pore space	0.3	0.67	0.20
								Porosity	0.3	0.86	0.26
								Organic carbon	0.3	0.52	0.16
				Stable Aggregates Index	0.2	0.82	0.16				
				Bulk density	0.2	0.83	0.17				
				Electrical conductivity	0.2	0.88	0.18				
Nutrient Cycling	**0.2**	**0.52**	**0.10**	**C cycle**	0.3	0.54	0.16	SOC	0.3	0.52	0.16
								Active Carbon	0.3	0.70	0.21
								ß-glucosidase	0.2	0.42	0.08
								Respiration	0.2	0.44	0.09
				N cycle	0.2	0.33	0.06	P.M.N.	0.4	0.09	0.04
								Leucine aminopeptidase	0.4	0.49	0.20
								Chitinase	0.2	0.49	0.10
				P cycle	0.2	0.59	0.11	P available	0.6	0.61	0.37
								alkaline phosphatases	0.4	0.56	0.22
				pH	0.075	0.78	0.06				
				C.E.C.	0.075	0.61	0.05				
				Water-filled pore space	0.075	0.67	0.05				
				Arylsulfatase (S cycle)	0.075	0.43	0.03				
Filtering and Buffering	**0.2**	**0.55**	**0.11**	**Toxicity**	0.3	0.77	0.23	Arsenic	0.2	0.79	0.16
								Lithium	0.4	0.83	0.33
								Thallium	0.4	0.69	0.28
				Biological activities and biodiversity	0.3	0.31	0.093	P.M.N	0.3	0.09	0.03
								Respiration	0.3	0.44	0.13
								QBS-ar	0.3	0.51	0.15
				DNA content	0.1	0.50	0.05				
				pH	0.1	0.78	0.08				
				SOC	0.2	0.52	0.10				
Biodiversity and Habitat	**0.2**	**0.39**	**0.078**	**Enzymes**	0.25	0.47	0.11	B-glucosidase	0.16	0.42	0.07
								alkaline	0.16	0.56	0.09
								hosphate	0.16	0.52	0.08
								acid phosphatase	0.16	0.49	0.08
								leucine aminopept	0.16	0.43	0.07
								arylsulfatase	0.16	0.49	0.08
								chitinase			
				DNA content	0.25	0.50	0.13				
				QBS-ar	0.25	0.51	0.13				
				P.M.N	0.25	0.09	0.02				
Environmental Protection and productivity		**Overall SQI**		**0.54**							

Table 35- Soil quality score card for P-S-R rotation system using linear transformed data of expert opinion-chosen MDS.

SOIL FUNCTION	WEIGHT	SCORE	VALUE	INDICATOR Level 1	WEIGHT	SCORE	VALUE	INDICATOR Level 2	WEIGHT	SCORE	VALUE
Physical Stability and Structural Support	**0.2**	**0.53**	**0.10**	Macroaggregate Stability	0.4	0.63	0.25				
				Mean Weighted Diameter	0.1	0.43	0.04				
				Organic carbon	0.3	0.40	0.12				
				Bulk density	0.1	0.75	0.08				
				Respiration	0.1	0.36	0.04				
Water relations	**0.2**	**0.73**	**0.14**	**Facilitate water movement and availability**	0.4	0.62	0.24	Water-filled pore space	0.3	0.70	0.21
								Porosity	0.3	0.97	0.29
								Organic carbon	0.3	0.40	0.12
				Stable Aggregates Index	0.2	0.79	0.16				
				Bulk density	0.2	0.75	0.15				
				Electrical conductivity	0.2	0.91	0.18				
Nutrient Cycling	**0.2**	**0.52**	**0.10**	**C cycle**	0.3	0.48	0.14	SOC	0.3	0.40	0.12
								Active Carbon	0.3	0.62	0.18
								ß-glucosidase	0.2	0.49	0.098
								Respiration	0.2	0.36	0.072
				N cycle	0.2	0.38	0.076	P.M.N.	0.4	0.35	0.14
								Leucine aminopeptidase	0.4	0.41	0.16
								Chitinase	0.2	0.39	0.08
				P cycle	0.2	0.60	0.12	P available	0.6	0.66	0.4
								alkaline phosphatases	0.4	0.50	0.2
				pH	0.075	0.77	0.06				
				C.E.C.	0.075	0.76	0.06				
				Water-filled pore space	0.075	0.70	0.05				
				Arylsulfatase (S cycle)	0.075	0.31	0.02				
Filtering and Buffering	**0.2**	**0.47**	**0.094**	**Toxicity**	0.3	0.51	0.15	Arsenic	0.2	0.42	0.08
								Lithium	0.4	0.61	0.24
								Thallium	0.4	0.46	0.18
				Biological activities and biodiversity	0.3	0.35	0.10	P.M.N	0.3	0.35	0.11
								Respiration	0.3	0.36	0.11
								QBS-ar	0.3	0.45	0.14
				DNA content	0.1	0.59	0.06				
				pH	0.1	0.77	0.08				
				SOC	0.2	0.40	0.08				
Biodiversity and Habitat	**0.2**	**0.46**	**0.092**	**Enzymes**	0.25	0.45	0.11	B-glucosidase	0.16	0.49	0.08
								alkaline hosphate	0.16	0.50	0.08
								acid phosphatase	0.16	0.72	0.12
								leucine aminopept	0.16	0.41	0.07
								arylsulfatase	0.16	0.31	0.05
								chitinase	0.16	0.39	0.06
				DNA content	0.25	0.59	0.15				
				QBS-ar	0.25	0.45	0.11				
				P.M.N	0.25	0.35	0.09				
Environmental Protection and productivity		**Overall SQI**		**0.52**							

3.11. Outcome comparisons

The soil quality index outcomes were considered using two-way ANOVA (Table 36). This analysis revealed that for all indexing method combinations, the ability of both PCA and EO methods based on linear scoring technique to discriminate among rotation systems was equal. However, within the additive and weighted indices, the PCA-chosen MDS based on non-linear scoring resulted in more significant differences among rotation systems than performed linear scoring method.

Table 36- Outcomes comparison for three different soil quality index calculations method using a two-way ANOVA (P-values for randomized complete block design) of rotation systems.

	Additive soil quality index			
	Linear scoring	Linear scoring	Non-linear scoring	Non-linear scoring
	EO[1]	PCA[2]	EO	PCA
Rotation	< 0.0001	< 0.0001	0.0439	0.0004
Replicate	0.0006	0.028	0.1937	0.1707
	Weighted soil quality index			
	Linear scoring	Linear scoring	Non-linear scoring	Non-linear scoring
	EO	PCA	EO	PCA
Rotation	< 0.0001	< 0.0001	0.005	< 0.0001
Replicate	0.033	0.012	0.35	0.025
	systematic soil quality index			
	Linear scoring	Non-linear scoring		
	EO	EO		
Rotation	< 0.0001	< 0.0001		
Replicate	0	0		

A comparison among the three soil quality indices (Table 37) shows that for most indexing method combinations, the P-S-R rotation obtained higher soil quality index values than the other rotation systems. Instead, the S-R-R showed the highest SQI values based on systematic soil quality index method. We suggest, therefore, that rice cultivation with alternating leguminous crops (e.g. pea, soybean) could result in higher overall soil quality than monoculture crop or fallow-rice rotation.

[1] EO represents minimum data set defined by expert opinion

[2] PCA represents minimum data set obtained from principal component analyses

These results corroborate the ideas of Karlen et al. (2006), who suggested that more diverse crop rotations would improve the sustainable agriculture. Furthermore, Aziz et al. (2011) showed Corn-soybean-wheat-cowpea rotations had higher soil quality values than corn-corn and corn-soybean. Their results implied that multiple cropping systems could be more effective for maintaining and enhancing soil quality than sole-cropping systems. In addition, Abdollahi et al. (2014) found that winter-spring crop rotations resulted in the highest soil structural quality and consequently the highest crop yield compared to a winter crop rotation.

The results of additive and weighted indices using non-linearly scored EO MDS were different from each other; namely, R-R-R had the highest SQI based on additive index using non-linearly scored EO MDS whereas F-R received the highest SQI with regard to weighted additive index using non-linearly scored EO MDS (Appendix 6). However, no differences between additive and weighted additive indices using PCA MDS were observed for the relative SQI ratings of rotation systems. This finding is in agreement with Andrews et al. (2002 a), who showed that weighting the additive SQI does not change the relative SQI rankings for the vegetable production systems (Appendix 6).

The index outcomes were also compared, using the Pearson correlation coefficients, with particular soil indicators, and end –point variables, in order to understand the level of correspondence and the direction of alterations (see Table 38).This correlation analysis revealed that in most cases, the soil quality indices were strongly affected by organic carbon (%), pH, extractable phosphorous, DNA, and chitinase. For instance, weighted additive index using the linearly scored EO MDS was positively correlated with WFPS, OC, DNA and chitinase; while it had negative correlation with extractable phosphorous and pH. This could be expected since these soils have neutral to moderately alkaline pH and extractable phosphorous examined fell within the optimum levels, therefore the higher values of pH and phosphorous can lead to reduce soil quality. Instead, the additive index using the linearly scored EO MDS were not correlated with most of the investigated indicators (i.e. WFPS, WSA, P, OC, Ec, Zn, rice yields, Li and TI).

In some cases, there was no reasonable explanation between soil indicator and the SQI. For example, there was a significantly negative correlation between Zn concentration and systematic soil quality index using the scored EO MDS; our findings highlighted that zinc concentration in almost all rotations was lower than the optimum range. However, a significantly positively correlation was observed between Zn concentration and weighted additive index using the PCA MDS.

The management goals demonstrated significant correlations with the indices. The systematic soil quality index based on linear scoring method was highly inversely correlated with Li and TI; it was obvious since our results demonstrated the soils of study area to be highly contaminated by this metals. Furthermore, rice yields were significantly positively correlated with weighted soil quality index; these relationships endorse the SQI outcomes. These results disagree with the results of Andrews et al. (2002a) who reported that management goals have fewer significant correlations with the indices in vegetable production systems. However, our findings confirm the findings of research reported by Liu et al. (2014) indicating that rice productivity had positive correlation with soil quality index. Outcome comparisons considering the results of two-way ANOVA and correlations point to almost all soil quality indices using the non linear scored PCA-MDS as being slightly more representative of overall soil quality in the paddy fields.

Table 37- A comparison among rotation systems for the calculated soil quality indices using a two-way ANOVA at P = 0.05, with LSD test; same lowercase letters in columns represent that the soil quality index does not differ between rotation systems.

Rotation	**Expert Opinion**					
	Additive Index		*Weighted additive index*		*systematic soil quality index*	
	Non linear	Linear	Non linear	Linear	Non linear	Linear
R-R-R	0.74 a	0.51c	0.51 b	0.40 d	0.51c	0.47 c
S-R-R	0.72 b	0.54 b	0.48 c	0.41 c	0.59 a	0.54 a
F-R	0.72 b	0.56 b	0.54 a	0.43 b	0.57 b	0.54 a
P-S-R	0.71 b	0.62 a	0.52 ab	0.50 a	0.48 d	0.52 b
	Principal Components Analysis					
	Additive Index		*Weighted additive index*			
	Non linear	Linear	Non linear		Linear	
R-R-R	0.63 c	0.69 c	1.63 b		1.77 b	
S-R-R	0.62 c	0.68 c	1.55 c		1.73 c	
F-R	0.65 b	0.71 b	1.59 b		1.78 b	
P-S-R	0.72 a	0.76 a	1.94 a		2.01 a	

Table 38- Pearson Correlation coefficients for three different soil quality index between some particular soil indicators and management goal variables.

Variable	Additive SQI				Weighted SQI				systematic SQI	
	Linear scoring		Non-linear scoring		Linear scoring		Non-linear scoring		Linear scoring	Non-linear scoring
	EO	PCA	EO	PCA	EO	PCA	EO	PCA	EO	EO
BD(g cm^{-3})	-0.30*	-0.24	-0.12	-0.24	-0.27	-0.18	-0.15	-0.21	-0.18	-0.21
Water filled pore space (%)	0.03	0.65**	0.78**	0.57**	0.56**	0.51**	0.74**	0.42**	-0.08	0.22
WSA cw (%)	0.09	0.53**	0.22	0.45**	0.22	0.26	0.21	0.24	0.09	0.10
PH	-0.37**	-0.32*	-0.63**	-0.21	-0.55**	-0.09	-0.48**	-0.01	-0.12	-0.15
P (mg kg^{-1})	-0.18	-0.43**	-0.68**	-0.49**	-0.54**	-0.35*	-0.72**	-0.42**	-0.01	-0.52**
OC (%)	0.18	0.46**	0.55**	0.42**	0.45**	0.35*	0.55**	0.30*	0.23	0.24
EC (dS m^{-1})	0.01	0.12	0.20	0.15	0.25	0.01	0.24	0.08	0.37*	0.54**
Zn	0.03	0.25	0.06	0.30*	0.01	0.53**	0.20	0.51**	-0.84**	-0.53**
DNA	0.39**	0.64**	0.79**	0.55**	0.62**	0.41**	0.69**	0.32*	0.14	0.21
Chitinase	0.36*	0.43**	0.47**	0.32*	0.52**	0.23	0.31*	0.10	0.17	-0.18
Yield 2010	0.01	0.28	0.15	0.27	0.39**	0.38**	0.27	0.36*	-0.64**	-0.27
Yield 2011	-0.17	0.32*	0.31*	0.34*	0.27	0.36*	0.41**	0.40**	-0.11	0.65**
Yield 2012	-0.21	0.19	0.26	0.22	0.05	0.17	0.31*	0.22	0.31*	0.94**
Li	-0.22	0.24	0.07	0.30*	-0.12	0.51**	0.23	0.52**	-0.64**	-0.05
Tl	-0.01	-0.01	-0.24	-0.01	-0.18	0.17	-0.16	0.13	-0.47**	-0.58**

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

3.12. Paddy field characteristics in IRRI

3.12.1. Effect of different cropping patterns and fertilizer treatments on soil chemical and biological properties

Table 39 provides the descriptive statistics of measured soil chemical and biological properties including pH, EC, Active C , P.M.N, basal respiration (BR), the cumulative value of soil respiration during 1, 4, 7, 14, 21, and 28 days of incubation (Ct) and metabolic quotient (q $CO_{2)}$ for each rotation system and fertilizer treatment.

The pH value did not change significantly among rotation systems (5.82 to 6.46, i.e. always moderate acid). In other words, crop rotation and fertilizer treatments did not demonstrate any significant effect on soil pH. However, the soil electrical conductivity varied significantly in reply to management systems; it ranged from 1.65 to 2.86 dS m^{-1}, the latter being beyond the salinity threshold reported by FAO (1976). Significant positive correlation was observed for soil pH with basal respiration ($r = 0.32$, $p < 0.05$) and EC ($r = 0.5$, $p < 0.01$) (Table 45). A wide range of values was found for potentially mineralizable nitrogen (26.11 to 141.58). The flooded rice-flooded rice rotation system and conventional N management presented the highest P.M.N, while rice-maize and zero N management had the lowest P.M.N. A significant positive correlation was observed for P.M.N with EMI. Active carbon of soil samples varied from 1417.10 to 1352.91 mg kg^{-1}. The highest active carbon (1417.10 mg kg^{-1}) was observed in the soils under R-R and zero N management, and the lowest (1352.9 mg kg $^{-1}$) found in rice-maize under conventional N management. There was a significantly negative correlation between the active C values with the cumulative value of soil respiration (Ct), whereas no significant correlation was found between active C and other soil parameters.

Among four fertilizer treatments, conventional N showed the highest (0.84µg C-CO_2 g^{-1} h^{-1}) and the lowest (0.011) basal respiration at the 28th day of incubation (CV= 92.59).

Table 39- Descriptive statistics of some chemical and biological soil parameters in the study area (IRRI, the Philippines).

Rotations		**Soil parameters**						
		pH	Ec (dS m^{-1})	Active c (mg kg^{-1})	P.M.N (mg g^{-1} week)	BR28[1]	Ct[2]	qco_2[3]
R-M	Mean	6.02	1.86	1384.53	72.73	0.75	55.62	0.002
	Max	6.16	2.11	1415.69	116.29	0.15	65.87	0.004
	Min	5.89	1.65	1352.91	33.25	0.01	40.44	0.0003
	CV (%)	1.59	7.52	2.31	33.79	68	14.31	50
FR – NR	Mean	6.36	2.17	1385.17	65.12	0.31	106.41	0.008
	Max	6.56	2.45	1416.74	102.30	0.84	146.72	0.022
	Min	6.28	2	1353.06	36.89	0.08	72.53	0.0019
	CV (%)	0.0095	7.11	2.29	33.39	90.32	24.86	87.5
R-R	Mean	6.28	2.36	1385.94	93.83	0.21	79.84	0.005
	Max	6.41	2.86	1417.10	141.58	0.40	102.51	0.01
	Min	6.11	1.91	1354.58	43.63	0.071	65.89	0.001
	CV (%)	0.015	13.5	2.29	31.12	42.38	13.17	46
Fertilizer treatment								
Conventional N	Mean	6.25	2.07	1385.13	79.61	0.27	81.02	0.007
	Max	6.42	2.86	1416.77	141.58	0.84	146.72	0.022
	Min	6.02	1.65	1352.91	43.47	0.011	46.38	0.0003
	CV (%)	2.24	15.46	2.27	37.39	92.59	36.70	85.71
Zero N	Mean	6.20	2.19	1385.30	73.05	0.13	80.23	0.0036
	Max	6.46	2.64	1417.10	126.54	0.24	136.58	0.0064
	Min	5.89	1.79	1353.57	26.11	0.036	40.44	0.0009
	CV (%)	3.06	11.87	2.26	36.07	53.84	30.32	52.77
Conventional N +rice residues	Mean	6.09	1.76	1384.51	74.13	0.12	93.51	0.003
	Max	6.28	2.21	1415.86	98.09	0.27	137.39	0.007
	Min	5.89	1.57	1353.81	50.88	0.045	70.26	0.0011
	CV (%)	2.46	12.5	2.28	18.14	60.16	22.36	59.37
Zero N + rice residues	Mean	6.11	1.75	1385.16	74.84	0.20	78.43	0.005
	Max	6.39	1.88	1415.90	116.29	0.44	111.01	0.011
	Min	5.88	1.64	1354.50	33.25	0.049	55.29	0.0013
	CV (%)	2.61	4.97	2.28	34.39	60	22.92	58.49

[1] Basal respiration at the 28th day of incubation (µg C-CO_2 g^{-1} h^{-1})
[2] The cumulative value of soil respiration(mgr C- CO_2/ kg.day) (during 1,4, 7, 14, 21, and 28 days of incubation)
[3] Metabolic quotient (1000) mg C-CO_2/(mg act C)/ Kg/h

Table 40- Descriptive statistics of some soil physical properties (IRRI, the Philippines).

Rotations		Indices of soil stability							
		SAI (%)	SmaI (%)	WSAs (%)	WSAcw (%)	MWDs (mm)	MWDcw (mm)	GMDs (mm)	GMDcw (mm)
R-M	Mean	77.15	49.79	63.75	66.89	0.94	1.33	0.69	0.74
	Max	91.24	84.47	73.08	77.20	1.67	1.99	0.81	0.86
	Min	61.38	31.06	53.33	50.66	0.76	0.88	0.61	0.48
	CV (%)	11.96	35.02	9.30	10.55	24.74	24.81	81.15	13.51
FR – NR	Mean	71.47	45.76	66.14	70.01	0.94	1.79	0.69	0.85
	Max	99.28	82.23	97.68	85.97	1.67	3.87	1.25	1.39
	Min	28.09	13.96	51.97	56.18	0.76	1.14	0.02	0.67
	CV (%)	28.66	43.96	19.50	10.06	24.72	41.89	39.13	21.17
R-R	Mean	72.84	48.78	65.83	70.11	1.66	21.40	0.80	0.91
	Max	95.33	87.27	85.08	87.22	3.67	3.88	1.36	0.64
	Min	51.17	24.80	54	57.45	0.69	0.89	0.58	1.44
	CV (%)	22.43	45.73	15.20	12.77	57.83	42.52	38.75	24.11
Fertilizer treatment									
Conventional N	Mean	72.61	45.91	66.72	69.72	1.44	1.84	0.77	0.85
	Max	95.33	87.27	97.68	87.22	3.67	3.88	1.36	1.44
	Min	28.09	13.96	51.97	56.18	0.71	1.02	0.59	0.48
	CV (%)	26	44.97	18.33	11.64	61.11	46.73	27.27	25.88
Zero N	Mean	75.04	50.32	63.76	68.29	1.27	1.67	0.68	0.82
	Max	99.28	84.47	76.09	79.36	3.11	2.91	1.04	1.08
	Min	56.27	24.80	54	50.66	0.69	0.88	0.02	0.61
	CV (%)	16.44	37.18	10.35	10.76	49.60	40.11	29.41	17.07
Conventional N +rice residues	Mean	75.59	50.19	67.06	72.36	1.68	1.91	0.80	0.90
	Max	96.72	84.66	75.20	86.25	3.35	3.61	1.07	1.24
	Min	49.67	23.24	52.96	63.11	0.74	0.99	0.59	0.70
	CV (%)	21.19	39.39	11.51	10.07	59.52	50.26	21.25	23.33
Zero N + rice residues	Mean	79.74	52.93	67.63	71.17	1.73	2.01	0.81	0.90
	Max	95.17	79.56	78.57	82.19	3.37	3.42	1.14	1.23
	Min	56.01	27.49	57.01	54.83	0.74	0.95	0.60	0.67
	CV (%)	15.61	35.51	11.50	12.73	58.38	47.26	23.45	22.22

Basal respiration varied significantly in response to rotation systems; flooded rice-non flooded rice had the highest BR. This same trend was observed for Ct and qCO_2. In general, the low

qCO_2 associated with high amounts of active carbon may indicate that soils under flooded rice-non flooded rice are under a good health status. This hypothesis was supported by the results of soil biological quality index (QBS-ar) (see subsection *3.12.4.).* In other words, a high value of active carbon demonstrates that the liable fraction of soil C that is readily available as a source of carbon and energy for the soil microbes can increase soil microbial activity (i.e. more decomposition and plant nutrient cycling) and consequently improve soil quality (Aziz et al., 2011; Islam and Weil, 2000).

3.12.2. Effect of different cropping patterns and fertilizer treatments on the indices of soil stability

Table 40 present the results of indices of soil stability (see chapter 2), the results revealed that four fertilizer treatment and 3 different rotation systems had a significant impact on soil aggregate size stability. Flooded rice-non flooded rice presented the highest (99.28 %) and lowest (28.09 %) SAI. Moreover, the highest SAI (%) was found in the soils under zero N treatment, while the lowest amount of SAI (%) observed in conventional N.

However, the soils under flooded rice –flooded rice and conventional N treatment had the highest SMAI (87.27). This same trend was observed for MWDs, MWDcw, WSAcw, GMDs, and GMDcw. Our results do not support the outcome of a study by Young Jung et al. (2011), on nitrogenous fertilizer effects on soil structural properties, who found that with less N fertilizer higher proportion of macroaggregates values were obtained than with more N treatments. Contrary to expectation the results of correlation analysis revealed that the stable aggregate index values were negatively affected by soil basal respiration ($r = -40$, $p < 0.01$) (Table 40). However, most of indices of soil stability had positive correlation with each other (see table 39).

3.12.3. Soil biological quality Index (QBS-ar)

The QBS-ar values and the classes of soil biological quality in the examined soil samples ranged from 0 to 72 and 0-6 respectively. The highest QBS-ar values were found, in flooded rice-non flooded rice under conventional N and rice-maize rotation under zero N treatment respectively. Furthermore, maximum diversity of microarthropod groups was observed in the specified rotations. Although flooded rice-flooded rice under conventional N and rice-maize cropping pattern under conventional N+ rice residues presented a low soil biological quality (i.e. class of soil quality = 0) in comparison with other management systems. It is likely that crop residues and soil microclimatic parameters (e.g. moisture condition) are responsible for the recorded varieties in the QBS-ar values (Aziz et al, 2011; Pagliai et al., 2004). Figure 22 shows some microarthropod groups that were found under the study area (IRRI).

Fig. 22. Some of the microarthropod groups that were found under study area.

Table 41- Pearson correlation coefficients between some measured soil parameters (IRRI).

	SAI	SMAI	MWDs	MWDcw	WSAs	WSAcw	GMDS	GMDwc	PH	EC	PMN	ActiveC	EMI (QBS-AR)	BR28	Ct	qCo2
SAI (%)	1.00															
SMAI (%)	0.81**	1.00														
MWDs (%)	0.45**	0.75**	1.00													
MWDcw (%)	0.28*	0.63**	0.93**	1.00												
WSAs (%)	0.39**	0.58**	0.68**	0.65**	1.00											
WSAcw (%)	0.22	0.48**	0.75**	0.75**	0.62**	1.00										
GMDS (%)	0.39**	0.67**	0.87**	0.83**	0.68**	0.69**	1.00									
GMDwc (%)	0.27*	0.61**	0.92**	0.95**	0.66**	0.87**	0.83**	1.00								
PH	-0.02	0.14	0.36**	0.40**	0.20	0.26*	0.19	0.37**	1.00							
EC (dS m^{-1})	-0.11	0.01	0.09	0.19	-0.02	-0.03	0.02	0.11	0.51**	1.00						
PMN (mg g^{-1} week^{-})	0.11	0.25	0.18	0.25	0.22	-0.03	0.16	0.14	0.08	0.22	1.00					
ActiveC (mg kg^{-1})	0.48**	0.35**	0.21	0.15	0.44**	0.31*	0.21	0.22	0.01	0.01	-0.10	1.00				
EMI (QBS-AR)	-0.16	-0.16	-0.09	-0.08	0.06	-0.02	-0.04	-0.05	-0.02	-0.13	-0.41*	-0.02	1.00			
BR28(μg C-CO_2 g^{-1} h^{-1})	-0.40**	-0.30*	-0.08	-0.03	-0.13	-0.05	-0.10	-0.01	0.32*	0.12	0.04	-0.23	-0.23	1.00		
Ct[1]	-0.34**	-0.33**	-0.09	-0.08	-0.27*	-0.09	-0.17	-0.06	0.28*	0.09	-0.07	-0.41**	-0.13	0.64**	1.00	
qCo2 [2]	-0.40**	-0.31*	-0.10	-0.05	-0.14	-0.07	-0.12	-0.03	0.31*	0.11	0.05	-0.23	-0.31	1.00**	0.63**	1.00

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

[1] The cumulative value of soil respiration(mgr C- CO2/ kg.day) (during 1,4, 7, 14, 21, and 28 days of incubation)

[2] Metabolic quotient (1000) mg C-Co2/(mg act C)/ Kg/h

Table 42- Eco-morphological index, EMI, and QBS-ar values for 3 different cropping systems under four fertilizer treatments in wet season after field preparation in June 2013.

System	Sub-treatment	Biological forms	Number	Abundance	EMI	QBS-ar	Class of SQ
Rice-Maize	Conventional N	Coleoptera	2	28.57	10	25	1
		holometabolous (larvae)	4	57.14	10		
		Hymenoptera	1	14.28	5		
Rice-Maize	Zero N	Acarina	1	10	20	62	6
		Collembola	1	10	20		
		Coleoptera	1	10	20		
		Diptera	5	50	1		
		Hymenoptera	1	10	1		
		Nematoda	1	10	-		
Rice-Maize	Conventional N+ Rice residues	Hymenoptera	1	1	25	3	0
		Hemiptera	1	1	25		
		Diptera	2	1	50		
Rice-Maize	Zero N+ rice residues	Acarina	1	12.5	20	47	2
		Coleoptera	2	25	20		
		Diptera	2	25	1		
		Hymenoptera	2	25	5		
		Hemiptera	1	12.5	1		
Rice+ Aerobic Rice	Conventional N	Acarina	1	11.11	20	72	6
		Collembola	1	11.11	20		
		Coleoptera	2	22.22	20		
		Hymenoptera	3	33.33	1		
		holometabolous (larvae)	1	11.11	10		
		Diptera	1	11.11	1		
Rice+ Aerobic Rice	Zero N	Collembola	2	66.66	20	21	3/2
		Hemiptera	1	33.33	1		
Rice+ Aerobic Rice	Conventional N+ Rice residues	Coleoptera	1	8.33	20	27	2
		Hymenoptera	3	25	5		
		Hemiptera	1	8.33	1		
		Diptera	7	58.33	1		
Rice+ Aerobic Rice	Zero N+ rice residues	Coleoptera	**2**	18.8	20	47	3
		Collembola	**1**	9.09	20		
		Diptera	2	18.8	1		
		Hemiptera	3	25	**1**		
		Hymenoptera	3	25	5		
Rice+Rice	Conventional N	Hymenoptera	2	50	5	6	0
		Diptera	2	50	1		
Rice+Rice	Zero N	Coleoptera	3	60	20	26	2
		Hymenoptera	1	20	5		
		Diptera	1	20	1		

Chapter four

4.1. Conclusions

A wide range of soil attributes including physical, chemical and biological properties was measured in order to assess soil health status using five soil functions (physical stability and structural support, water relations, nutrient cycling, filtering and buffering, biodiversity and habitat).

We examined several methods for selecting a minimum data set (MDS), transforming the indicators, and calculating indices using data from the Veneto region, Italy, in a paddy soil with four different rotation systems (rice-rice-rice: R-R-R; soya-rice-rice: S-R-R; fallow-rice: F-R; pea-soya-rice: P-S-R) and three replications in April (after field preparation, field moist condition), June (mid-tillering, the early period of waterlogging), August (panicle formation, the late period of waterlogging) and October (after harvesting, drained soil condition) over the 2012 growing season.

Nine MDS indicators were chosen from 62 physical, chemical and biological soil attributes using principal component analysis. The five principal components accounted for 85.96% of the total variance in the total data set. However, ten MDS indicators were used based on expert opinion (i.e. soil management assessment framework: SMAF).

Minimum data set indicators were scored by both linear and non-linear scoring techniques. Scoring results revealed that the non-linear scoring could represent the performance of most indicators better than did the linear scoring method while the linear scoring method outcomes were dependent on measured range.

The results of stepwise regression did not show any significant difference between the EO and PCA methods (for both observed and non-linear scored MDSs, as independent variables) in reflecting variability of management goals (or end points, as dependent variables). Our

findings highlighted that Extractable phosphorous (mg kg^{-1}), B-glucosidase, and water filled pore space were the main factors limiting 2011-2012 rice yield when using the EO-MDSs as the independent variable , whereas the 2010 yield was strongly explained by the scored PCA-MDSs including AWC, Zn, CEC and chitinase.

Finally, the indices (namely, an additive index, a weighted additive index and systematic soil quality index) were calculated by integrating indicator scores obtained either by expert opinion or principal component analysis. In general, for most indexing method combinations, P-S-R rotation received statistically higher SQI values than the other rotation systems. However, the results of systematic soil quality index based on non-linear scoring method showed that S-R-R yielded the highest SQI, followed by F-R, R-R-R and P-S-R. The weighted additive index using PCA MDS was considered redundant for rating SQI in this rice-based production system, since weighting additive SQI did not change the relative SQI ratings for the rotation systems.

All soil quality indices proved to be suitable for assessing the effects of various cropping patterns on soil functions. However, almost all SQ indices obtained based on the non-linearly scored PCA-MDS, proved to be significantly better than the other SQ indices calculated by other methods in evaluating soil quality. We conclude, therefore, that a little number of indicators, for example a MDS of 9 out of the 62 indicators or 10 indicators as suggested by SMAF, supplied information adequately on soil quality distinctions among the rotation systems. Furthermore, a limited number of indicators could allow reducing the cost of the analyses for evaluating soil quality for further research in management of paddy fields at the study area.

Future studies on the effects of cropping patterns in rice-based production systems are recommended. Several questions remain unanswered at present: whether applying those few indicators would be useful in other rotation systems, or what would be the effect of various

rotation systems (i.e. rice-maize, rice-barley or rice-wheat) on the other soil functions. In addition, more research work is needed in the indicator interpretation step (i.e. developing scoring curve algorithms) since it is the most complex and the main phase in the evaluation of soil quality.

Our results relating to soil enzymes demonstrated that enzyme activities varied with rotation systems and growth stages due to changing moisture regime, pH, soil organic carbon and crop rotation in paddy soil. In contrast to earlier findings, our findings highlighted that the activity of all considered soil enzymes in P-S-R and R-R-R rotations was increased at the early period of waterlogging compared with field moist soil condition, although waterlogged soil condition had an inhibitory effect for all enzymes in other rotation systems (i.e. S-R-R and F-R).

Compared with field moist soil, drained soil condition resulted in a significant increase ($P < 0.05$) of B-glucosidase, arylsulfatase, alkaline and acid phosphatases, leucine aminopeptidase (except of fallow-rice), and chitinase activities in all rotations, while compared with drained soil, early waterlogging (in month of June) significantly decreased ($P<0.05$) B-glucosidase, alkaline and acid phosphatases, leucine aminopeptidase (except of pea-soya-rice), arylsulfatase, chitinases. Our results suggest that the activity of most enzymes decreases in the different experimental conditions with the following order: drained soil > late waterlogged > early waterlogged > moist soil.

Soil organic-C was positively correlated with acid and alkaline phosphatases, and arylsulfatase while ß-glucosidase, chitinase and leucine aminopeptidase were not significantly correlated to soil organic-C. Enzyme activities were always correlated among them.

However, the response of enzymes to waterlogging differed with the chemical species and the cropping pattern. The best rotation system for chitinase, B-glucosidase and leucine

aminopeptidase activity (C and N cycles) proved R-R-R (i.e. monoculture continuous system), while for alkaline and acid phosphatases and arylsulfatase (P and S cycles) it was the S-R-R (i.e. alternate cropping patterns of *gramineae* and legumes). This suggests that, in agreement with current literature, there may be soil characteristics (e.g. OC, pH, texture) unique to each rotation system that improve the activity of specific enzymes.

Therefore, adequate soil management with R-R-R rotation could be useful in C- and N-deficient soils, while S-R-R rotation could enhance enzyme activities in P-and S-deficient soils, and thus improve soil quality and crop yield.

Further studies on the effects of different cropping patterns on enzyme activity in paddy soils are needed in order to better understand soil-crop relationships and to improve crop yield.

Our findings regarding the total soil and plant elements highlighted that the total concentration of most of the measured metals (i.e. Al, As, Ba, Be, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sb, Si, Ti, Zn, , and V) in the soil samples were lower than those of control (the reported background values of soils in Veneto region), and therefore the study area is not contaminated by them. However, Li and TI presented higher concentration than those of control and the Italian threshold limits in all rotation. Although Sn (in all rotation) concentration overcame the permissible limit values according to Italian legislation (D.L. 152/2006) for green and residential areas, it is likely related to the geochemistry of the parent material.

Only low quantities of metals were taken up into the upper parts of plants, and non essential elements (As, Cr, Ni, Pb) were accumulated in roots more than in soil and in the aerial parts, regardless of the rotation system. The root probably acts as a barrier to the translocation of metals within the plant. The phenomenon is thus responsible for various metal levels in distinct parts of plants. The study evidenced that rice could be considered as an excluder plant for Li, Sn,

Tl. Therefore, rice plants (*Oryza sativa* L.) have a good potential to be used in contaminated-sites restoration projects, in areas contaminated with As, Ba, Zn by the phytoextraction technique. Conversely, Cr, Cu and Ni are likely available for phytostabilization. Moreover, since rice is able to accumulate non essential metals especially in root or leaf parts, but not in the edible part, there is very limited hazard for human population consuming rice crops.

Concerning the IRRI experiments, only some indicators (e.g. soil respiration, active C, P.M.N, EC, pH, aggregate stability) could be determined, the whole data set being not available.

Based on available data, our observations associated with the results of paddy fields in IRRI indicated that both the cropping patterns and fertilizer treatments had a significant impact on the most measured soil quality indicators. It seems that flooded rice-flooded rice cropping systems and conventional N fertilizer management have a higher soil health condition than other treatments.

In conclusion, our experiments demonstrate that the best management practice should be selected based on environmental conditions and particularly climate. In fresh temperate climate with alternate seasons like north Italy the best rotation system proved alternating legumes with rice with conventional fertilization. Conversely with warmer and more humid climate conditions like the Philippines the best management proved flooded rice with conventional N fertilization.

However, further indicators should be measured to calculate the soil quality index value, in order to understand the real soil health condition in the study area.

Chapter five

5.1. References

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English

The thesis title is: ***Investigation of Soil Health and Sustainable Management in Rice-Based Production Systems***

Abstract

Interest in evaluating soil quality in agricultural systems has been developing since improper soil and crop management decisions resulted in resource degradation, and subsequent detrimental changes in soil functions. A wide range of soil attributes, including physical, chemical and biological properties, were measured in the paddy fields of the Veneto region, Italy over the 2012 growing season (i.e. in April after field preparation, field moist condition; June mid-tillering, the early period of waterlogging; August panicle formation, the late period of waterlogging; October after harvesting, drained soil condition). The paddy soils were under four different rotation systems (rice-rice-rice: R-R-R; soya-rice-rice: S-R-R; fallow-rice: F-R; pea-soya-rice: P-S-R) and three replications.

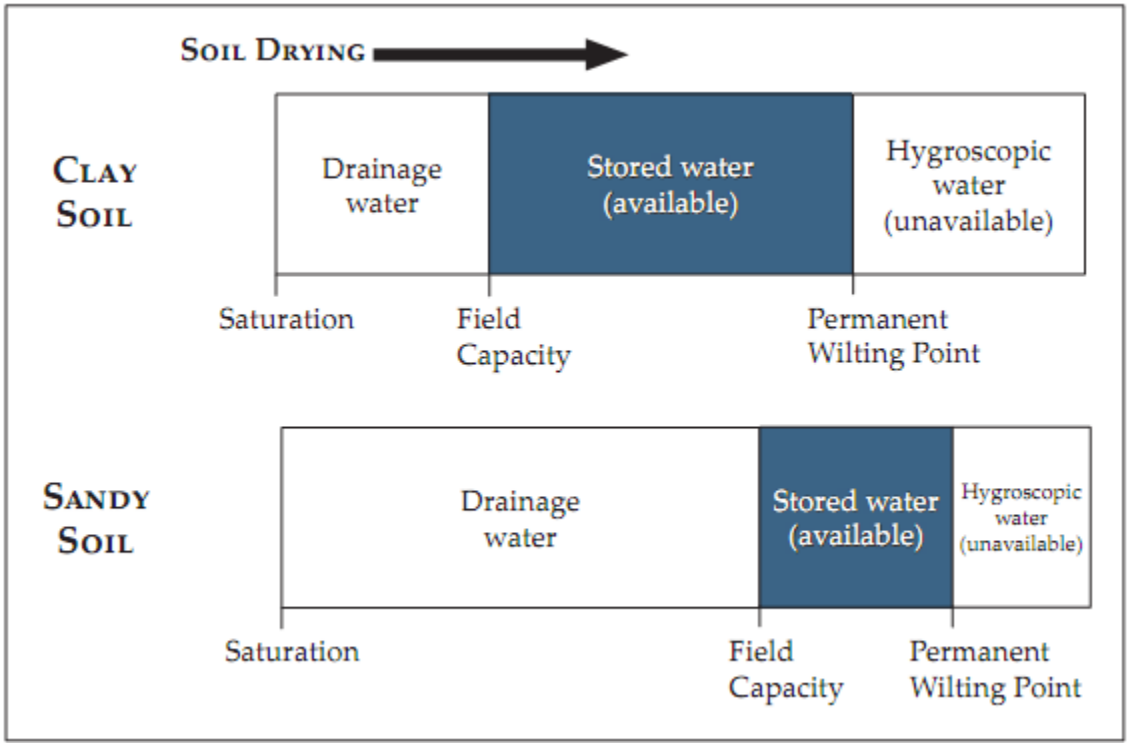
Soil quality was evaluated using three different soil quality indices, namely: an additive index, a weighted additive index and a systematic soil quality index, by integrating indicator scores (linear and non-linear) obtained either by expert opinion or principal component analysis. Another part of field and laboratory experiments were conducted at the International Rice Research Institute, research farm in Los Baños, Philippines, to evaluate the effect of three rotation systems (i.e. flooded rice – non-flooded rice, flooded rice – flooded rice, flooded rice – maize) and four fertilizer treatments (i.e. conventional N management, no rice residues; zero N, no rice residues; conventional N management, with rice residues; zero N, with rice residues) on some soil indicators, in order to have a comparison between two different environmental conditions.

All soil quality indices proved to be suitable for assessing the effects of various cropping patterns on soil functions. However, almost all SQ indices obtained based on the non-linearly scored PCA-MDS proved to be significantly better than the other SQ indices calculated by other methods in evaluating soil quality. The results of stepwise regression highlighted that extractable phosphorous (mg kg^{-1}), β-glucosidase, and water filled pore space were the main factors limiting 2011-2012 rice yield when using the EO-MDSs as the independent variable, whereas the 2010 yield was strongly explained by the scored PCA-MDSs including AWC, Zn, CEC and chitinase. In general, for most indexing method combinations, P-S-R rotation received statistically higher SQI values than the other rotation systems. However, the results of systematic soil quality index based on non-linear scoring method showed that S-R-R yielded the highest SQI, followed by F-R, R-R-R and P-S-R. We suggest, therefore, that rice cultivation with alternating leguminous crops (e.g. pea, soybean) could result in higher overall soil quality than monoculture crop or fallow-rice rotation. Our observations associated with the results of paddy fields in IRRI indicated that both the cropping patterns and fertilizer treatments had a significant impact on the most measured soil quality indicators. It seems that flooded rice-flooded rice cropping systems and conventional N fertilizer management have a higher soil health condition than other treatments.

Our experiments demonstrate that the best management practice should be selected based on environmental conditions and particularly climate. However, further indicators should be measured to calculate the soil quality index value in order to understand the real soil health condition in the study area.

Keywords: Paddy fields, rice, soil quality indices, Italy, the Philippines

Appendix 1: Water storage for Clay and Sandy soil (The blue shaded area represents water is available for plant use). Source: Gugino et al., 2007.



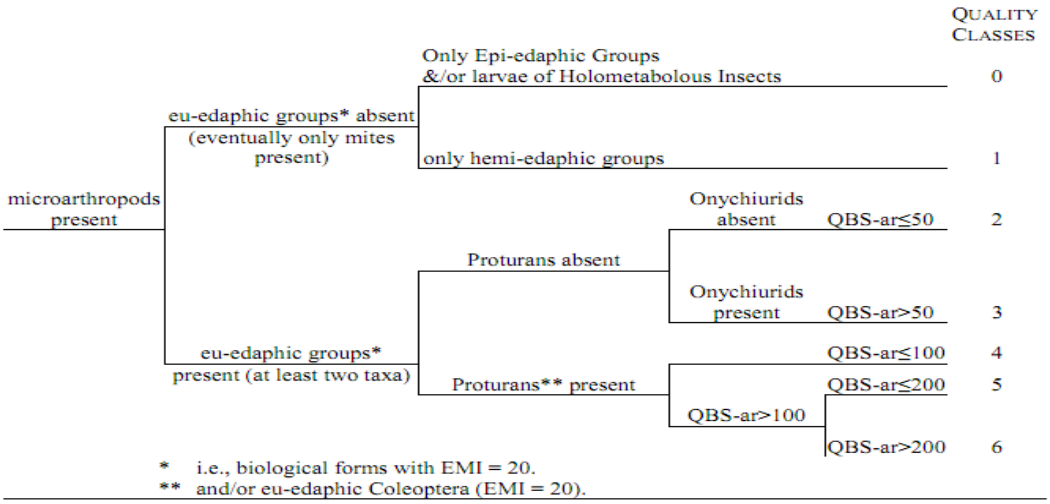
Appendix 2: Indices used for assessing soil stability

Index	Reference/ Comments
Mean Weight Diameter: $MWD = \sum_{i=1}^{n} \bar{x}_i w_i$	Van Bavel (1949)
Geometric Mean Diameter: $GMD = \exp\left[\sum_{i=1}^{n} w_i \log(\bar{x}_i) \Big/ \sum_{i=1}^{n} w_i\right]$	Mazurak (1950) $\bar{x}_I$ is the mean diameter of each size fraction, W is the proportion total sample weight occurring on the size fraction i.
Water Stable Aggregates: $WSA(\%\ \text{of soil} > 250\ \mu m) = \frac{\text{weight of dry aggregates} - \text{sand})}{(\text{weight of dry soil} - \text{sand})} \times 100$	Kemper (1996) and USDA (1998)
Stable Aggregates and Stable Macroaggregates Index: $SAI = \frac{\sum_{j=1}^{n} [(n+1) - j]S_j}{\sum_{j=1}^{n} [(n+1) - j]T_j}$ and	Ma´rquez et al. (2004) Based on size stability distribution, slaked and capillary wetted pretreatments, and subsequent-slake. Total sand correction. J=1 for the largest size class. m is the total number of size classes larger than 250 µm. S_j is the amount of stable aggregates in fraction j. T_j is total amount of aggregates in fraction j (from the capillary- wetted treatment). n is the total number of size fractions.
$SMaI = \frac{n\sum_{j=1}^{m} [(m+1) - j]S_j}{m\sum_{j=1}^{n} [(n+1) - j]T_j}$	SMAI defined as the ratio between the weighted average of the amount of stable macroaggregates (> 250 µm) and the total weighted average of all soil aggregates.

Appendix 3: Eco-morphological Indices EMIs. Source: Parisi et al. 2004

Group		Score
Protura		20
Diplura		20
Collembola		1-20
Microcoryphia		10
Zygentomata		10
Dermaptera		1
Orthoptera		1-20
Embioptera		10
Blattaria		5
Psocoptera		1
Hemiptera		1-10
Thysanoptera		1
Coleoptera		1-20
Hymenoptera		1-5
Diptera (larvae)		10
Other holometabolous insects	(larvae)	10
	(adults)	1
Acari		20
Araneae		1-5
Opiliones		10
Palpigradi		20
Pseudoscorpiones		20
Isopoda		10
Chilopoda		10-20
Diplopoda		10-20
Pauropoda		20
Symphyla		20

Appendix 4: Transformation of QBS-ar values into Soil Quality Classes. Source: Parisi et al., 2004.



Appendix 5: Background and reference values of metal concentrations (mg kg^{-1}) in soils and plants in agricultural lands.

	Soil (mean or range)	Reference	NGB[1]		Plant	Reference
Aluminium	10000 – 40000	Kabata-Pendias and Mukherjee (2007)	159000	Aluminium	> 3000	Kabata-Pendias and Mukherjee (2007)
Antimony	10	Legislation DM 152/2006	1.5	Antimony	0.002 – 0.029	Kabata-Pendias and Pendias (2001)
Arsenic	20	Legislation DM 152/2006	50	Arsenic	0.49 – 0.93	Liu et al (2005)
Barium	84 – 960	Kabata-Pendias and Mukherjee (2007)	456	Barium	2 – 13	Kabata-Pendias and Mukherjee (2007)
Cadmium	2	Legislation DM 152/2006	1.17	Cadmium	5.6 – 32[2]	Kabata-Pendias and Mukherjee (2007)
Chromium (total)	150	Legislation DM 152/2006	141	Chromium	0.01 – 0.35[2]	Kabata-Pendias and Pendias (2001)
Cobalt	20	Legislation DM 152/2006	20	Cobalt	5 – 270[3]	Kabata-Pendias and Mukherjee (2007)
Copper	120	Legislation DM 152/2006	79	Copper	0.3 – 13	Kabata-Pendias and Mukherjee (2007)
Iron	1000 – 28000[4]	Kabata-Pendias and Mukherjee (2007)	67100	Iron	17 – 50	Kabata-Pendias and Mukherjee (2007)
Lithium	4.2 – 14.18	Kabata-Pendias and Krakowiak (1995)	17	Lithium	15[5]	Kabata-Pendias and Mukherjee (2007)
Lead	100	Legislation DM 152/2006	46	Lead	<1[2]	Kabata-Pendias and Mukherjee (2007)
Manganese	50 – 9200	Kabata-Pendias and Mukherjee (2007)	1060	Manganese	27 – 50	Kabata-Pendias and Mukherjee (2007)
Nickel	120	Legislation DM 152/2006	125	Nickel	0.34 – 14.6[2]	Kabata-Pendias and Mukherjee (2007)
Silicon	7.5 – 940	Takeda et al(2004)	606000	Silicon	40000 – 100000	Alyoshin et al. (1988)
Strontium	5 – 1000	Kabata-Pendias and Mukherjee (2007)	320	Strontium	– 2.5 in grains 45 – 74 in leaves 219-662 aerial part	Kabata-Pendias and Mukherjee (2007)
Tin	1	Legislation DM 152/2006	3.7	Tin	0.2 – 1.9[6]	Kabata-Pendias and Pendias (2001)
Thallium	1	Legislation DM 152/2006	0.50	Thallium	0.05	Markert (1992)
Titanium	5000 – 10000	Kabata-Pendias and Mukherjee (2007)	7200	Titanium	1.2 – 7	Grabanov (1970)
Vanadium	90	Legislation DM 152/2006	89	Vanadium	0.01 0.7	Kabata-Pendias and Pendias (1999) and Anke et al. (2003)
Zinc	150	Legislation DM 152/2006	155	Zinc	18	Kabata-Pendias and Mukherjee (2007) and Chon and Lee (2004)

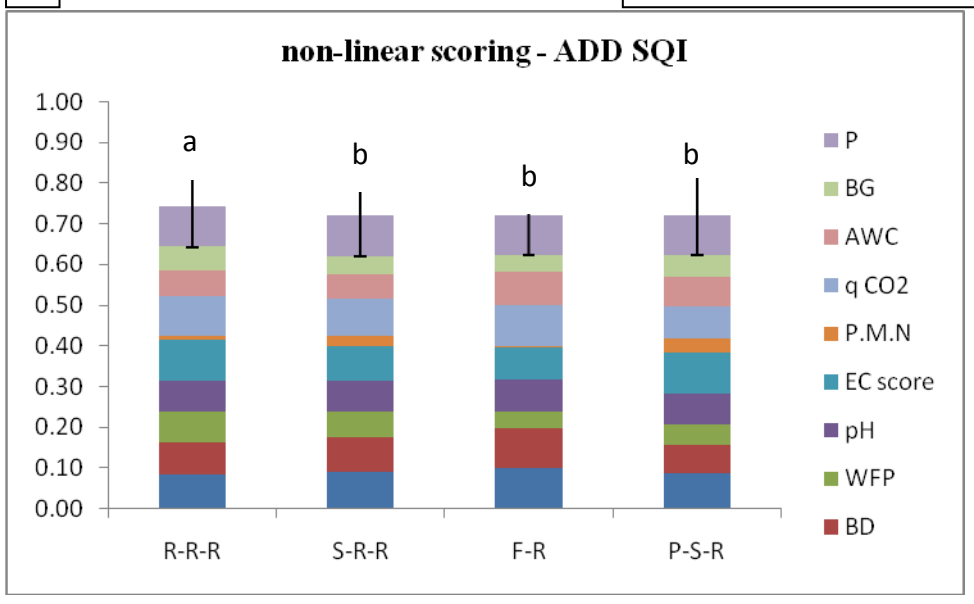
[1] The natural geochemical background level (Rudnick and Gao, 2004; ARPAV, 2011).
[2] Mean values for barley and wheat respectively
[3] Mean values in cereal grains
[4] Median for world soils (light sand: 0.1-1 and medium loamy: 0.8 – 2.8)
[5] High contents of Li for lettuce
[6] Mean values in grass

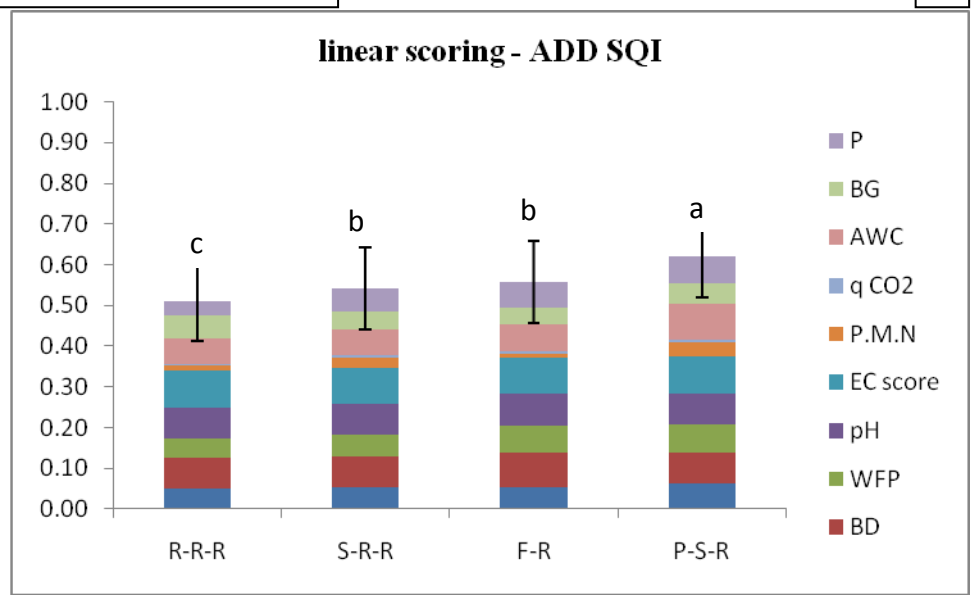
Appendix 6: Additive (ADD SQI) and weighted additive soil quality indices (WTD SQI) applying linear or non-linear scored indicators chosen by expert opinion or principal components analysis minimum data set (MDS) selection methods for different rotation systems (error bars represent ±1 S.D. from the mean SQI value for each treatment. Different lowercase letters indicate significant differences among mean values of rotation systems, at $\alpha = 0.05$, two-way ANOVA with LSD test.

a

Expert Opinion – selected MDSs

b





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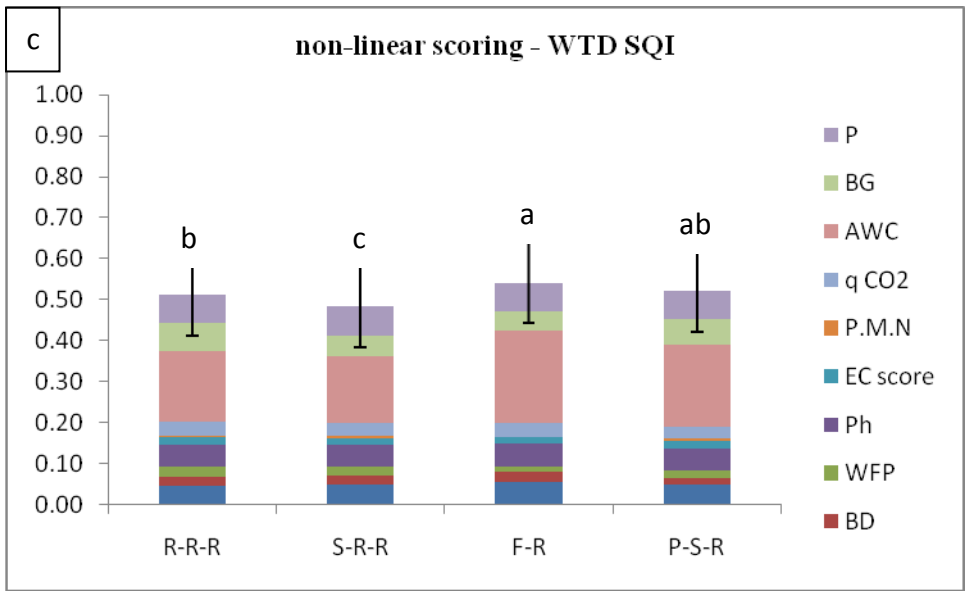

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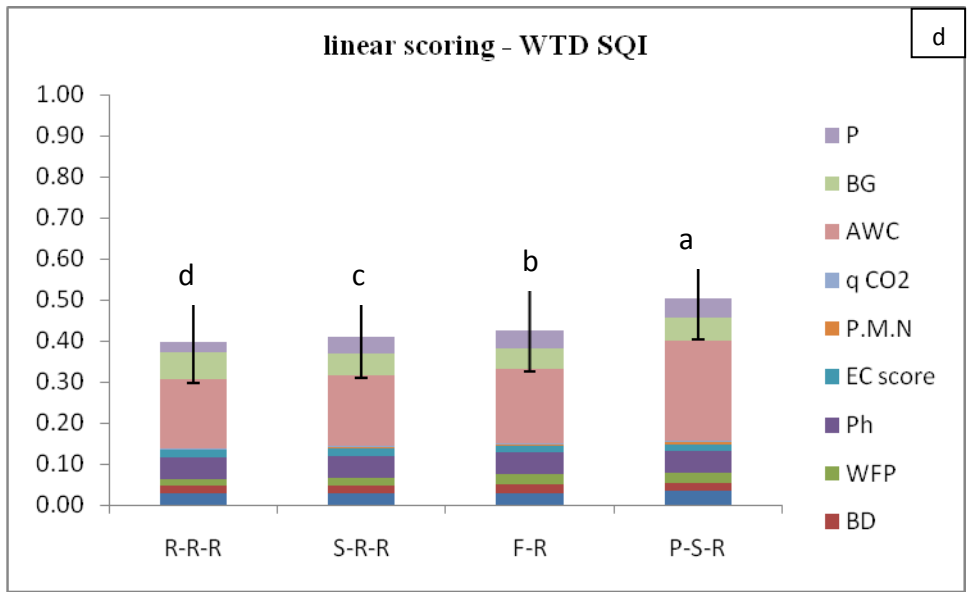
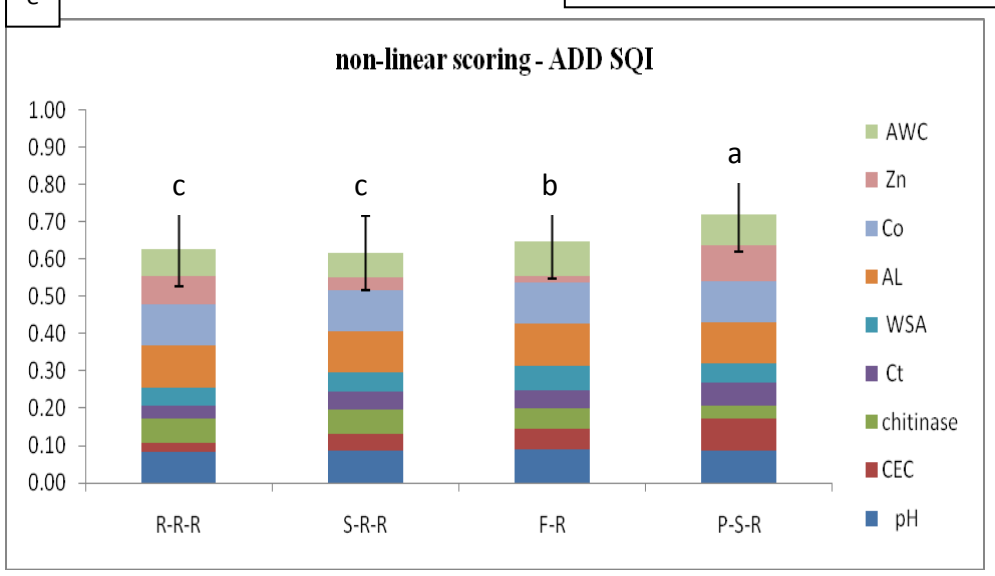


Principal Components Analysis-chosen MDSs

e



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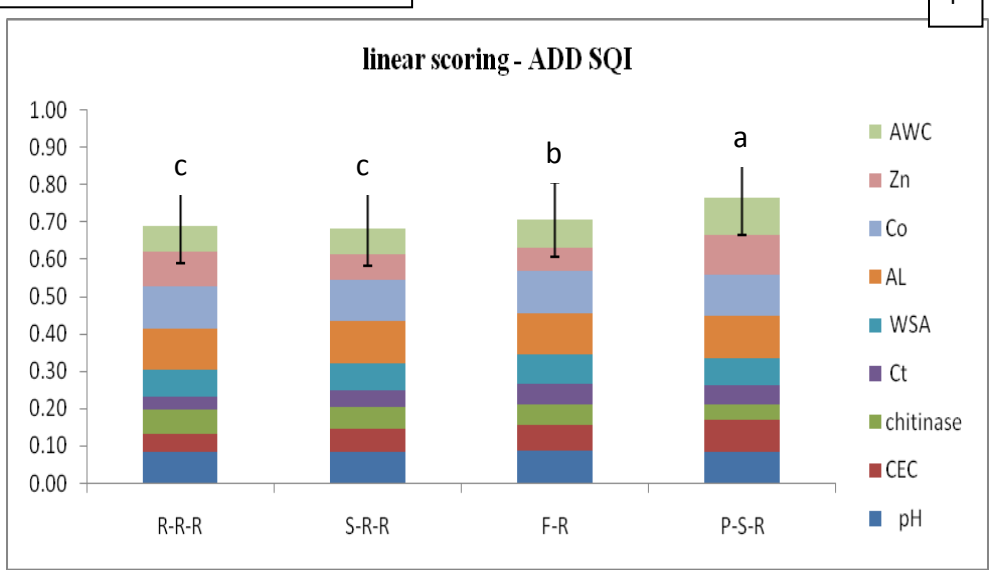

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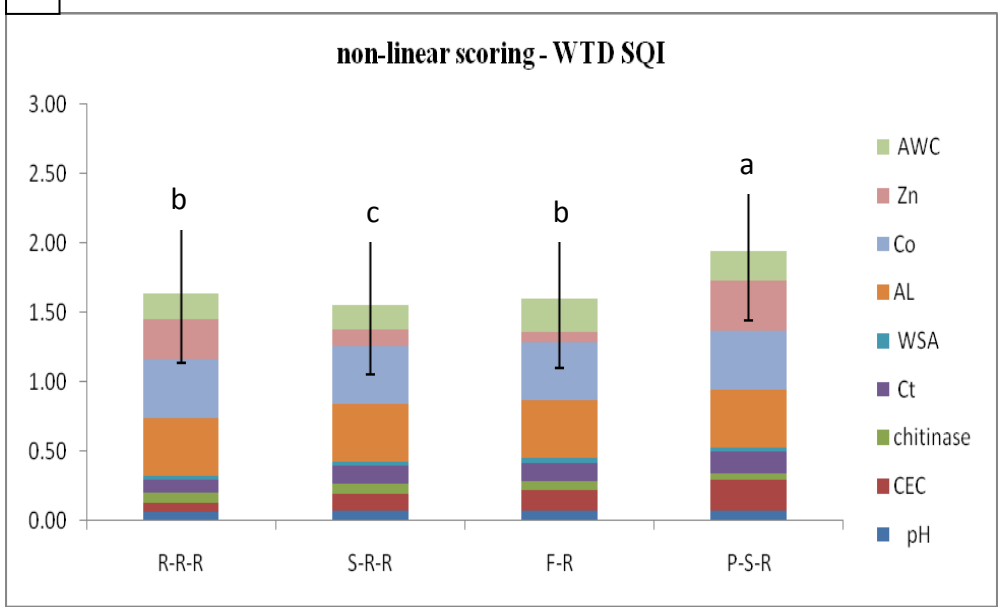

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