CAN TREATED WASTEWATER BE USED AS AN ALTERNATIVE WATER RESOURCE FOR AGRICULTURAL IRRIGATION? CHANGES IN SOIL AND PLANT HEALTH AFTER THREE YEARS OF MAIZE CULTIVATION IN WESTERN ANATOLIA, TURKEY

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Abstract. The limited availability of fresh water caused by climate change and global population increase has made research necessary into alternative water sources which are sustainable but of lower quality, in order to meet the demands of agriculture in arid and semi-arid areas. However, there are concerns that irrigation with treated wastewater (TWW) could lead to a deterioration of soil health and a decline in plant yield. The objective of the field study was to determine the effects of medium-term (3-year) irrigation with secondary TWW, fresh water (FW) and a mix of 1:1 TWW/FW (HW) on various microbiological and biochemical soil properties of a Typic Xerofluvent soil and on maize (*Zea mays* L.) productivity in Mediterranean climatic conditions. In line with this aim, microbial biomass C (MBC), basal soil respiration (BSR) and nitrogen mineralization (N_{min}), and the activities of the enzymes alkaline phosphatase (ALKPA), dehydrogenase (DHG), β-glucosidase (GLU), aryl sulphatase (ArSA) and urease (UA) were determined. In addition, the grain yield of maize was measured. Taking three-year averages into account, it was found that although all biological dependent variables measured in connection to the application of TWW had been affected in a range of 2% to -11% (mean -3%), the differences created by the use of FW did not reach the level of statistical significance. With the application of HW, a significant and more severe reduction of -19% ($P<0.05$) was found. The application of TWW increased only ALKPA and ArSA activity, by 4% and 2%, respectively, and this result was found not to be significant. Even though the effect of TWW on soil health was not found to be significant, a negative effect was found on the grain yield of maize plants ($P<0.05$) at a level of -17% (1.68 t ha^{-1}). The fact that no significant effect was found of the TWW used in this study conducted under Mediterranean climatic conditions on soil health is an indicator of its potential long-term use with different crops and different irrigation techniques. Furthermore, it has been proved that the re-use of treated wastewater, which has an effect close to that of fresh water, can be an important part of the integrated management of water resources, and is a reliable alternative water source, which can provide an effective solution in conditions of water shortage.
Keywords: *secondary treated domestic wastewater, microbial biomass carbon, soil enzymatic activity, nitrogen mineralization, principal component analysis*

Introduction

Water use has been increasing in the past hundred years at twice the rate of population growth, and in a growing number of areas, water supplies have reached their limits of sustainability. Worldwide water use by sector stands at 69% for agriculture, 19% for industrial use, and 12% for urban use. This distribution varies from one continent to another, but in regions other than Europe, agricultural use comes in first place (FAO, 2016). Due to the fact that the competition for water between different economic sectors increases, the re-use of wastewater is seen as an important component in an integrated water management policy.

Moreover, semi-arid and arid areas are particularly exposed to the impacts of climate change on fresh water. According to a consensus reached by climate change models, by

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the 2070s, a 100-year drought of today's magnitude will return, on average, more frequently than every 10 years in parts of Spain and Portugal, western France, the Vistula Basin in Poland, and western Turkey (Kundzewicz et al., 2007). Therefore, it is also important to reduce the pressure on freshwater sources by methods which will not cause damage to the ecosystem. In countries such as Turkey which are located in arid and semi-arid regions, the need for irrigation in agricultural production either because of a shortage in the annual total of precipitation or because of uneven distribution is the most important factor. One of the methods applied to reduce the pressure of agricultural irrigation on clean groundwater and surface water resources is to use wastewater which has been subjected to a process of purification treatment for the purpose of irrigation. Thus, in recent years, the recycling of wastewater has gained importance as a part of the water sources in agriculture in many countries suffering water shortages (Qadir et al., 2007; Pedrero et al., 2010). For example, in recent years, some Mediterranean countries have been suffering water shortages of increasing intensity because of global climate change causing frequent and long-lasting periods of drought. In summer, in particular, these areas experience extreme water needs and uneven demand on a sectoral basis (Libutti et al., 2018). Water shortage and drought are emerging as a serious problem not only in arid regions but also in other parts of the world where fresh water is plentiful (Bixio et al., 2006; EU, 2007; FAO, 2012). Indeed, Europe in general is faced with water shortage (EU, 2007), and half of European countries are under water stress (Bixio et al., 2006). It is estimated that over 40% of the world's population will face water stress or scarcity over the next 50 years, which is a serious requirement to achieve sustainable management options of water resources (WHO, 2006).

Turkey has an arid or semi-arid Mediterranean climate, with an annual average precipitation of 646 mm, or 501 billion m^3 of precipitation annually. In terms of distribution by sector, in 2012 the greatest proportion of Turkey's water resources, 73%, were taken by agriculture. The amount of usable water per person annually is approximately 1652 m^3 (MEU, 2016). Nowadays Turkey, where is experiencing water scarcity, may be poor in terms of water due to population growth. In particular, water shortage in Western Anatolia is having a serious effect on the local economy, which is largely dependent on agriculture. Furthermore, in the coastal agricultural areas of the Aegean region, groundwater is used for irrigation in an excessive and often uncontrolled way, and these depleted underground areas are affected by seawater intrusion, resulting in salination of irrigation water. In addition to this, Alaşehir, the district where the experiment was conducted, is rich in geothermal springs, with water up to 287 °C, the hottest in Turkey. For these reasons, the use of groundwater resources is limited, and water for use in agricultural irrigation is provided from drinking water sources. Also, it is seen that the problem of water pollution is growing along with the rapid increases in population and industrialization, and surface water which can be used for agricultural irrigation is being polluted by domestic and industrial wastewater and by agricultural activity. For these reasons apart from the water shortage due to drought, there is a need to find alternative water resources. To sum up, water shortage involves not just a physical shortage of water resources, but also a progressive deterioration in water quality in many areas, and a reduction in the amount of water which is safe to use (FAO, 2018).

There has been an increase in the number of studies on using water resources effectively, creating new water resources, setting up sensible policies for sectors with a demand for natural water sources and their use of water, reducing evapotranspiration by

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changes in farming practices, increasing yield and sustainability of crops and soil ecosystems in conditions of water shortage, harvesting rainwater effectively, and reducing the water footprint by rational approaches and raising awareness. These have been necessitated by changes in the hydrological balance in connection with the newly recognized factor of climate change and global warming. Treated wastewater is a sustainable source of irrigation water for agriculture: it can be used to solve the imbalance between water demand and supply, with importance not only in meeting crop water needs (Becerra-Castro et al., 2015) but also in encouraging soil microorganisms with its load of organic material (García-Orenes et al., 2015), in particular plant nutrient elements such as phosphorus, which support crop yield and other growth parameters (Abdoulkader et al., 2015). The extent to which all of these positive contributions of treated wastewater manifest themselves varies according to physicochemical characteristics (such as content of organic material which can easily be broken down biologically, nutrient concentration, pH and electrical conductivity (EC), characteristics of the soil and crops (Liang et al., 2014), measured biological parameters of the soil (Knapp et al., 2010), history of application (Libutti et al., 2018), and geographical climatic conditions (Kayikcioglu, 2012; Bei et al., 2018). The re-use of waste water not only provides significant quantities of irrigation water, but helps to conserve sources of drinking water (Pedrero et al., 2010; Agrafioti and Diamadopoulos, 2012). On the other hand, treated wastewater also contains significant amounts of inorganic materials such as heavy metals, boron and salts, which can have adverse effects on crop growth and soil characteristics (Kayikcioglu, 2012; Frenk et al., 2014). Although the use of wastewater is an ancient practice, it has not always been managed properly, nor have quality standards been adhered to, with the result that the potential for exposing agricultural products, soil and groundwater to potential dangers arising from toxic and pathogenic pollutants has increased (Aiello et al., 2007; Qadir et al., 2007; Balkhair et al., 2016). In addition, changes in the consumption needs of society have caused changes in the characteristics of wastewater. Along with this, although world attention has become focused on the amounts, efficiency of use and allocation of water, poor management of wastewaters and agricultural drainage have created serious water quality problems in many parts of the world and have exacerbated the water crisis (Biswas et al., 2012). The best guarantee of clean groundwater sources is healthy and good-quality agricultural soil. When the soil operates at the optimum level of biological productivity, it can perform its biodegradation function and its absorption and filtration functions at the highest levels of efficiency (Muñoz et al., 2009).

Little is yet known concerning the responses of the soil microbial community to the application of treated wastewater (Ma et al., 2015) because, as a review study found, most studies of irrigation with wastewater neglected its effect on soil microbiota (Becerra-Castro et al., 2015). In a previous study, it was shown that the soil microbial community was clearly stimulated by the application of wastewater (Iyyemperumal and Shi, 2007), but significant decreases in soil enzyme activities were seen in connection with short-term application to vertisol-type soils (Kayikcioglu, 2012). In the application of treated wastewater, an approach has been adopted of focusing on the lasting effects on soil microbial characteristics in the medium and long term (Iyyemperumal and Shi, 2007). This at the same time may help to prevent the stability of the microbial community exposed to large amounts of nutrients from irrigation with treated wastewater (Saison et al., 2006), from masking the actual condition of microbes in the soil (Ma et al., 2015). Certain works in this area have evaluated the accumulated effects

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on organic C content and microbial activity in soils irrigated with wastewater which originates from both municipal and agricultural over a long period (Kayikcioglu and Sahin, 2013; Liang et al., 2014; García-Orenes et al., 2015; Cui et al., 2016; Adrover et al., 2017), but there is little information concerning changes in microbial activities occurring in an irrigation period annually.

Of the enzymes considered in this study, urease, β-glucosidase, phosphatase and aryl sulphatase in the soil take part in the nitrogen, carbon, phosphorus and sulphur cycles, respectively. Dehydrogenase is an enzyme which acts, differently from the others, inside the cell, and its activity shows the total oxidative capacity of the microbial biomass. It would be expected that different organisms and metabolic pathways would be brought into operation with the varying amounts of organic materials and nutrients supplied when the land is irrigated with wastewater. An increase in the activity of some enzymes, such as hydrolytic and proteolytic enzymes, laccases, cellulases and phosphatase, has been reported in soils irrigated with treated wastewater (Elifantz et al., 2011; Morugán-Coronado et al., 2011; Adrover et al., 2012; Alguacil et al., 2012; Frenk et al., 2014). For this reason, it has been suggested that irrigation with wastewater could stimulate the activity of microorganisms in the biochemical cycle of elements such as C, N and P. However, the direction of stimulation may vary in connection with the physicochemical characteristics of the wastewater and the soil to which it is applied (Kayikcioglu, 2012). Furthermore, the amount of organic matter providing the stabilization of enzymes remaining active in the extracellular environment in soils must also be borne in mind in studies of applications of irrigation with wastewater (Trasar-Cepeda et al., 2008).

The purpose of this study was to describe the short and medium-term effects of TWW irrigation on soil microbial activity and maize plant yield. For this aim, irrigation was carried out with FW or TWW, and crop yield and microbial activity were measured periodically over a three-year period. Because the parameters of soil enzyme activities and soil respiration are much more sensitive than physical and chemical parameters of the soil to soil management changes, because they are an early warning signal of ecosystem perturbations, and because they are a widely used indicator of soil quality (Friedel et al., 2000; Caravaca et al., 2002; Speir, 2002; Gianfreda et al., 2005; Falkowski et al., 2008; Roohi et al., 2017), such microbiological and biochemical parameters as N-mineralization, microbial biomass-C, basal soil respiration, and the enzymatic activities of dehydrogenase, alkaline phosphatase, β-glucosidase, urease and aryl sulphatase were used in this study. An additional aim was to determine the relationship between any changes in soil microbial populations and biochemical and microbiological changes caused by the type of irrigation water. Considering the importance of the soil microbiota on soil health and productivity, it is thought that this study will provide an important contribution to filling a gap in the literature.

Materials and methods

Study area

The field experiment was conducted in the experimental area (longitude: 28°31′36″ E; latitude: 38°22′24″ N; 152 m above mean sea level) of Alaşehir Vocational School of Manisa Celal Bayar University in Alaşehir which is a town and district of Manisa Province in the Aegean region of Turkey during the years between May 2011 - October 2013 (*Fig. 1*). In this study, the soil is classified as a Typic Xerofluvent (GDRS, 2001;

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Soil Survey Staff, 2010) and has a loam texture consisting of 21.6% clay, 30% silt, and 48.4% sand. The physicochemical properties of the experimental field at the beginning of the experiment are given in *Table 1*.

Figure 1. *Map shows the location of Alaşehir Vocational School of Manisa Celal Bayar University experimental area in the Manisa Province, Turkey*

Table 1. *Some physicochemical properties of the experimental site before the experiment*

Parameter	Mean[a]		Parameter	Mean[a]	
$pH_{(H_2O)}$	7.84	(0.04)[b]	Fe^{d} (mg kg^{-1})	10.80	(1.82)
Salinity (dS m^{-1})	0.877	(0.20)	Cu^{d} (mg kg^{-1})	2.04	(0.21)
Carbonates (g kg^{-1})	49.58	(1.65)	Zn^{d} (mg kg^{-1})	2.19	(0.35)
$C_{(Org)}$ (g kg^{-1})	14.52	(3.29)	Mn^{d} (mg kg^{-1})	14.85	(1.02)
C/N	8.85	(1.37)	Zn^{e} (mg kg^{-1})	87.18	(2.85)
$N_{(Kjeldahl)}$ (g kg^{-1})	1.62	(0.18)	Cu^{e} (mg kg^{-1})	34.90	(1.36)
$P_{(Bingham)}$ (mg kg^{-1})	5.90	(0.38)	Cr^{e} (mg kg^{-1})	21.94	(1.67)
K^{c} (mg kg^{-1})	519.4	(57.35)	Cd^{e} (mg kg^{-1})	0.61	(0.03)
Ca^{c} (mg kg^{-1})	2917	(55.51)	Pb^{e} (mg kg^{-1})	12.56	(0.21)
Mg^{c} (mg kg^{-1})	1185	(31.10)	Ni^{e} (mg kg^{-1})	48.59	(0.78)
Na^{c} (mg kg^{-1})	36.53	(20.14)	Co^{e} (mg kg^{-1})	15.78	(0.42)

[a]: Each value is the mean of three replicates of control soils; **[b]:** Standard deviation; **[c]:** NH_4OAc extract; **[d]:** DTPA extract; **[e]:** Total, HCl+ HNO_3 extract

Climatic pattern

The site is characterized by a Mediterranean semiarid climate with a long-term (January 1975 to December 2013) mean annual precipitation of 440 mm, a mean annual temperature of 16.8 °C and a temperature range of 6.6 °C (January) to 27.8 °C (July), according to the meteorological station in Alaşehir near the experimental area (Anonymous, 2017). In our study, the average annual air temperature (16.1 °C) and the average annual rainfall (442 mm) were similar to the long-term average climatic patterns *(Fig. 2)*. The lowest mean annual precipitation was 396.4 mm in 2011, and the highest mean annual precipitation was 493.6 mm in 2012 where the highest monthly precipation was with 133.6 mm in January. The highest mean annual temperature of 16.6 °C was recorded in 2012, and the lowest mean annual temperature was 15.4 °C in 2011.

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Minimum and maximum temperatures during the three growing seasons ranged from +4°C to 28.6 °C recorded on January 2012 and July 2012, respectively.

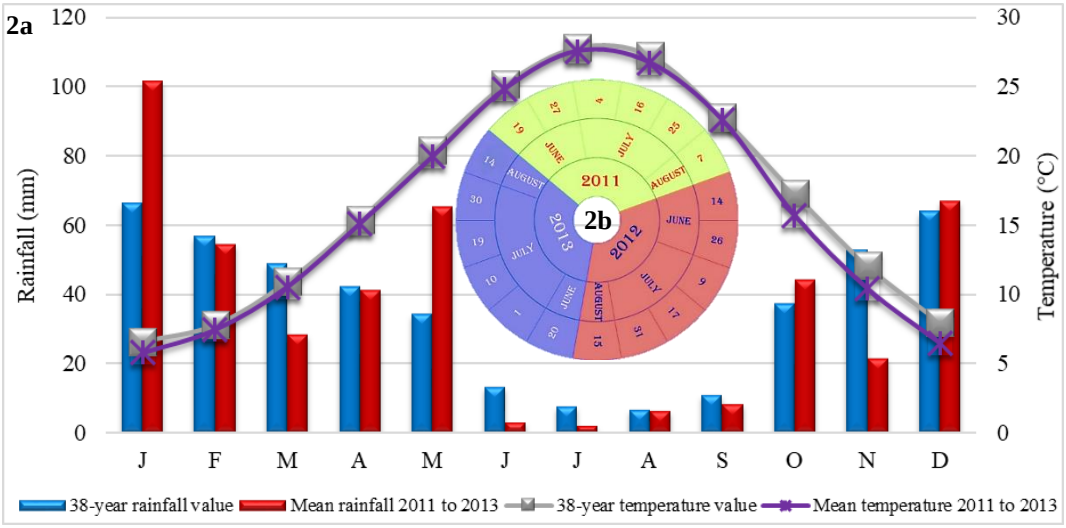

Figure 2. *a: Rainfall and temperature during experimental period (2011 to 2013); b: The circular chart in the middle of the graph shows the irrigation periods*

Irrigation water

The experiment consisted of three irrigation treatments with two water types: fresh water (FW), treated wastewater (TWW), and half of both two waters (HW). The TWW were received from the nearby secondary treatment plant in Alaşehir town of Manisa city in each year. The Alaşehir Wastewater Treatment Plant that receives the amount of sewage originating from industrial sources is low, serves around 36471 inhabitants with an influent flow rate of about 13392 m^3 day^{-1} in the year of 2011. Treated effluent is disinfected by chlorination and is discharged (0.155 m^3 sn^{-1}) to Alaşehir River in Gediz Basin which covers approximately 1703394 ha area and constitutes 2.2% of the overall surface area of Turkey (MEU, 2017). The main chemical properties of the two water of which samples were taken during 18 irrigation activities that were done six times a year during the 3-year trial period, are presented in *Table 2*. The values of different parameters analyzed in TWW were below the limit values allowed by FAO and Turkish directive, which regulates the use of wastewater in agricultural soil (Ayers and Westcost, 1994).

Experimental design

The experimental layout was a randomized-plot design with a total amount of 9 plots, with each plot measuring 3 m x 3.40 m. Three treatments were used (three replicates per treatment) as fresh water (FW), treated wastewater (TWW) and 50% FW+50%TWW (HW). Total water application rate was 540 mm for each irrigation season depending on the climate, the soil and the water requirements of the crop. Irrigation was made six times at a rate of 90 L m^{-2} during summer and autumn in each year. The irrigation water was applied by filling the plots with water up to the desired amount without overtopping the levees and the water retained there was allowed to infiltrate into the soil. Just before the experiment, the natural herbaceous vegetation,

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which emerged due to the fact that the experimental field was empty for two years, was eliminated and soil tilled at 20 cm.

Table 2. *Mean values of some properties of irrigation waters used in the experiment*

Parameter	FW[b]		TWW[c]		Parameter	FW		TWW	
pH	7.83	(0.09)[a]	7.44	(0.08)	NO_3-N (mg l^{-1})	0.458	(0.092)	0.076	(0.155)
EC (dS m^{-1})	0.60	(0.09)	1.47	(0.16)	NH_4-N (mg l^{-1})	bdl		0.899	(0.561)
Na (me l^{-1})	1.07	(0.44)	3.25	(0.99)	Cd (mg l^{-1})	bdl		bdl	
K (me l^{-1})	0.08	(0.01)	0.66	(0.18)	Pb (mg l^{-1})	bdl		bdl	
Ca+Mg (me l^{-1})	4.83	(0.58)	10.75	(1.36)	Ni (mg l^{-1})	0.001	(0.000)	0.003	(0.001)
Cl (me l^{-1})	1.33	(0.61)	3.51	(1.30)	Co (mg l^{-1})	bdl		bdl	
CO_3 (me l^{-1})	bdl[d]		bdl		Cr (mg l^{-1})	0.029	(0.010)	0.005	(0.004)
HCO_3 (me l^{-1})	4.16	(0.75)	10.30	(1.66)	Fe (mg l^{-1})	0.032	(0.015)	0.108	(0.038)
SO_4 (me l^{-1})	0.44	(0.19)	0.82	(0.26)	Cu (mg l^{-1})	0.013	(0.011)	0.028	(0.037)
SAR[e]	0.68	(0.27)	1.41	(0.44)	Zn (mg l^{-1})	0.012	(0.006)	0.055	(0.021)
RSC[f] (me l^{-1})	0.23	(0.18)	1.25	(0.91)	Mn (mg l^{-1})	0.008	(0.004)	0.102	(0.043)
IWQC[g]	C_2S_1		C_3S_1						

[a] Standard deviation, n=18; [b]: fresh water; [c]: treated wastewater; [d]: below detection limit; [e]: sodium adsorption ratio; [f]: residual sodium carbonate; [g]: irrigation water quality class, according to a method formulated by the U.S. Salinity Laboratory (1954)

Maize (*Zea mays* L. var. Kuadro) was planted in mid-May and was harvested about 147 days after sowing by hands depending on the climatic conditions in the year of 2011, 2012 and 2013. Nitrogen, phosphorus and potassium fertilizers were applied to all plots before the plantation as a fertilizer at the rate of 220 N kg ha^{-1} (as 15-15-15, NPK and 46% urea), 105 kg P_2O_5 ha^{-1} (as 15-15-15, NPK) and 105 kg K_2O ha^{-1} (as 15-15-15, NPK) in each year.

Composite soil samples (0–20 cm), composed of 5 cores per plot, were taken 15 days (first sampling: S_1- at the beginning of the experiment), and 132 days (second sampling: S_2- at harvesting) after maize seed sowing in the year of 2011, 2012 and 2013. Field-moist soil was sieved (2 mm) and divided into two subsamples. One was immediately stored at 4°C in plastic bags until microbiological and enzymatic activities were assayed (Öhlinger, 1995), and the other was air dried prior to physicochemical analysis. Microbial activities of fresh soils were determined within 30 days of sample collection. Water samples were collected during each irrigation activity in the irrigation season of the experiment. Some water samples were allocated with the addition of HCl for heavy metal and trace element analyses, while others were directly stored at 4 °C in the refrigerator (APHA-AWWA-WEF, 2005).

Soil physicochemical analyses

Soil pH, electrical conductivity, organic matter concentration, calcium carbonate were determined according to Thomas (1996), Rhoades (1996), Nelson and Sommers (1996), Loeppert and Suarez (1996), respectively. Particle size analysis of the soil was performed with the hydrometer method (Gee and Or, 2002). Total N was determined by Kjeldahl method according to Bremner (1996). Available P in soil was extracted by distilled water and determined using the molybdenum blue method (Bingham, 1962). Available K was measured by flame photometry after 1 mol L^{-1} $CH_3CO_2NH_4$ neutral extraction (Helmke and Sparks, 1996). The available metals (Fe, Cu, Zn and Mn) in the soil were analyzed according to the procedure described by Lindsay and Norvell (1978) using a

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diethylamine-pentaacetic acid (DTPA) solution. For determination of heavy metals, the soils were extracted with 3 parts HCl + 1 part HNO_3. The concentrations of Pb, Ni, Cr, Co , Hg and Cd in the extracts were determined by atomic absorption spectrometry (AAS) (ISO, 1995; 1998).

Soil biological analyses

Microbial biomass carbon (MBC) was determined by fumigation of the sample with ethanol-free $CHCl_3$ and extraction with 0.5 M K_2SO_4, according to Vance et al. (1987). Prior to analysis, samples were incubated for 12 h at 25°C. Basal soil respiration (BSR) was determined by the titration method (Isermeyer, 1952; Jäggi, 1976). Soil samples were incubated in a closed vessel at 25°C. The CO_2 produced was absorbed in sodium hydroxide and quantified by titration. N-mineralization (N_{min}) was assayed according to the method of Keeney (1982). This method involves the incubation of a soil sample under waterlogged conditions at 50 °C. At the end of 7 days, NH_4-N released from the soil water mixture was determined by modified Bertholet reaction.

Dehydrogenase activity (DHG, EC 1.1) was measured using the modified method of Thalmann (1968). Soil samples were suspended in a triphenyl tetrazolium chloride solution and incubated for 16 h at 25 °C. The triphenyl formazan (TPF) product was extracted with acetone and measured photometrically at 546 nm. Alkaline phosphatase activity (ALKPA, EC 3.1.3.1) was measured using the method of Eivazi and Tabatabai (1977). After the addition of a buffered P-nitrophenyl phosphate solution (pH 11), soil samples were incubated for 1 h at 37 °C. The P-nitrophenol (p-NP) released by phosphomonoesterase activity was extracted and colored with sodium hydroxide, and measured photometrically at 400 nm. β- Glucosidase activity (GLU, EC 3.2.1.21) was measured in accordance with the method of Hoffmann and Dedekan (1965). Using β-glucosido-saligenin (salicin) as a substrate, soil samples were incubated for 3 h at 37°C. Saligenin released from the substrate was determined colorimetrically at 578 nm after coloring with 2,6-dibromchinon-4-chlorimide. Arylsulphatase activity (ArSA, EC 3.1.6.1) was measured using the method of Tabatabai and Bremner (1970). After the addition of a P-nitrophenyl sulphate solution, soil samples were incubated for 1 h at 37 °C. P-nitrophenol (p-NP) released from the substrate was determined colorimetrically at 430 nm. Urease activity (UA, EC 3.5.1.5) was assayed according to the method of Kandeler and Gerber (1988). After the addition of a buffered urea solution, soil samples were incubated for 2 h at 37 °C. Released ammonium was extracted with potassium chloride solution and determined by a modified Bertholet reaction photometrically at 660 nm.

Water analyses

The sampling and analytical methods were performed following American Public Health Association (APHA) water sampling and analytical methods (APHA-AWWA-WEF, 2005). The test bottles (1000 ml) were filled with irrigation water samples, labeled, corked and then transferred to the laboratory for chemical analysis according to the standard tests. The tests were replicated three times for each sample point and then recorded. The samples tested for the following parameters: pH, electrical conductivity, Na, K, Ca+Mg, Cl, CO_3, HCO_3, SO_4, NO_3-N, NH_4-N, Cd, Pb, Ni, Co, Cr, Fe, Cu, Zn, Mn, residual sodium carbonate (RSC) and sodium absorption ratio (SAR).

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Statistical analysis

All data were tested for normality and homogeneity of distribution, and were log-transformed if required prior to analyses. The data was adjusted to compare the effects of different irrigation waters, and for this purpose, a MANOVA test was performed (*Supp. Tables 1 and 2*). The significance was tested between treatments for each sampling, as well as between samplings for each treatment to see changes over time and between treatments. The separation of means was carried out according to the average posthoc Duncan test $P<0.05$, assuming equal variance. The standard deviations (SD) which is a measure that is used to quantify the amount of variation or dispersion of a set of data values, were also calculated. Pearson correlation analyses were performed on all the chemical, microbiological and biochemical data. A standardized principal component analysis (PCA) was used to identify possible relationships between our tested soil biological indices (*Supp. Tables 3, 4 and 5; Supp. Fig. 1*). All statistical analyses were performed with the IBM SPSS Statistics 20.0.

Results and discussion

Evaluation with regard to microbiological parameters

Microbial biomass carbon (MBC)

Microbial biomass is a useful store of nutrients in the soil such as C, N, P and S, and an indicator of biogeochemical cycles (Lagomarsino et al., 2009). Namely it is an important and active soil component in the cycle of nutrients in the soil and which is responsible for the breakdown of organic pollutants. Microbial biomass, which is the total amount of immobilized C in microbial cells, has an extremely important function in maintaining soil fertility, as it is responsible for the degradation plant and animal wastes and for the immobilization and mineralization of plant nutrients. Microbial biomass consists of dormant and metabolically active organisms, and constitutes an early warning of changing soil conditions and the direction of change (Schloter et al., 2003). Because of this characteristic, it is the biological parameter which is most easily and fastest affected by agricultural management practices. In this study also, the MBC parameter was significantly affected by the independent variables. A significant effect was detected on the MBC value of 5% by the independent variable “treatments” in the experiment and of 1% by all other independent variables (*Supp. Table 2*). In an investigation of the effectiveness of treatments performed on a yearly basis, no significant differences were found (*Table 3*). Examining the mean value of MBC amounts (147.14 – 168.48 μg C_{mic} g^{-1}) determined a total of six times during the course of the experiment (*Fig. 3*), it was seen that the highest MBC value was with the application of FW. However, even though higher MBC values were obtained with FW, a close statistical grouping was found with TWW. The lowest activity was obtained with the application of HW.

On the other hand, the qualitative and quantitative size of the local microbial population of the soil determined the effect of the use of wastewater. Thus, in a study conducted in Spain, the mean MBC value in areas where TWW was not used was 508 μg C_{mic} g^{-1}, while in areas where TWW was applied, this value rose to 658 μg C_{mic} g^{-1}. Therefore, it is probable that there will be a difference in the reactions to applications shown by a microbial population which is numerically high. Similarly, it has been seen in many studies that MBC values increase as a result of irrigation with wastewater.

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Table 3. *The effect of different irrigation waters on some soil microbiological and biochemical parameters*

Parameter	Treatment	Year											
		2011				2012				2013			
		S_1[l]		S_2[m]		S_1		S_2		S_1		S_2	
MBC[a] *µg C_{mic} g^{-1}*	***HW***[i]	284.1	***A***	75.0	b ***CD***	192.3	b ***B***	57.8	b ***D***	167.9	***b B***	105.7	a ***C***
	TWW[j]	332.9	***A***	130.5	a ***C***	162.3	c ***BC***	64.8	b ***D***	221.7	a ***B***	44.7	b ***D***
	FW[k]	330.8	***A***	68.7	b ***CD***	233.9	a ***B***	93.2	a ***C***	227.2	a ***B***	57.1	b ***D***
BSR[b] *µg CO_2-C g^{-1} h^{-1}*	***HW***	2.46	***C***	6.06	b ***B***	5.93	***B***	6.91	b ***AB***	7.94	***A***	6.24	b ***B***
	TWW	2.59	***C***	7.52	ab***AB***	6.39	***B***	7.01	ab***AB***	8.40	***A***	7.36	ab***AB***
	FW	3.22	***C***	8.05	a ***AB***	6.36	***B***	7.18	a ***AB***	9.14	***A***	7.18	a ***AB***
N_{min}[c] *µg NH_4-N g^{-1} h^{-1}*	***HW***	15.64	***A***	6.53	c ***C***	9.52	***B***	10.51	b ***B***	3.83	b ***D***	4.24	***b D***
	TWW	15.07	***A***	10.99	b ***B***	10.99	***B***	12.88	a ***AB***	4.46	b ***C***	5.63	a ***C***
	FW	16.50	***A***	13.26	a ***B***	12.51	***B***	13.47	a ***B***	5.52	a ***C***	6.02	***a C***
ALKPA[d] *µg p-NP g^{-1} h^{-1}*	***HW***	405.9	***A***	370.0	***AB***	342.3	b ***BC***	288.7	b ***D***	372.1	b ***AB***	299.2	b ***CD***
	TWW	512.0	***A***	448.0	***BC***	435.9	a ***BC***	396.1	a ***CD***	462.5	a ***AB***	369.1	a ***D***
	FW	554.9	***A***	466.9	***B***	424.4	a ***B***	332.6	b ***C***	436.0	ab ***B***	302.8	b ***C***
DHG[e] *µg TPF g^{-1} h^{-1}*	***HW***	8.79	b ***A***	3.29	b ***CD***	3.36	b ***CD***	2.64	b ***D***	4.40	b ***B***	3.94	b ***BC***
	TWW	12.13	ab ***A***	4.67	a ***C***	5.05	a ***C***	5.11	a ***C***	7.56	a ***B***	4.40	ab ***C***
	FW	13.07	a ***A***	4.57	a ***C***	5.17	a ***C***	4.36	a ***C***	6.91	a ***B***	5.16	a ***C***
GLU[f] *µg Saligenin g^{-1} h^{-1}*	***HW***	25.11	b ***A***	18.55	b ***BC***	16.01	b ***CD***	13.47	***D***	20.02	b ***B***	18.17	***BC***
	TWW	27.15	ab ***A***	24.37	a ***A***	19.61	ab ***B***	15.80	***C***	24.81	a ***A***	19.35	***B***
	FW	28.78	a ***A***	25.84	a ***A***	20.63	a ***B***	16.20	***C***	25.50	a ***A***	19.47	***BC***
ArSA[g] *µg p-NP g^{-1} h^{-1}*	***HW***	125.5	b ***B***	122.6	b ***B***	192.5	b ***A***	170.6	***A***	121.9	b ***B***	81.6	***C***
	TWW	169.6	a ***C***	178.0	a ***BC***	248.2	a ***A***	214.9	***AB***	146.6	ab***CD***	113.4	***D***
	FW	169.8	a ***C***	165.0	ab ***C***	250.4	a ***A***	203.4	***B***	164.9	a ***C***	100.5	***D***
UA[h] *µg N g^{-1} h^{-1}*	***HW***	12.20	b ***C***	8.84	b ***DE***	11.35	b ***CD***	20.88	***B***	7.58	b ***E***	25.17	***A***
	TWW	17.97	a ***B***	11.25	ab***CD***	13.21	ab ***C***	17.08	***B***	9.44	a ***D***	24.26	***A***
	FW	18.62	a ***B***	12.43	a ***CD***	16.00	a ***BC***	16.95	***BC***	8.17	ab ***D***	24.81	***A***

[a]: Microbial biomass carbon; [b]: Basal soil respiration; [c]: Nitrogen mineralization; [d]: Alkaline phosphatase enzyme activity; [e]: Dehydrogenase enzyme activity; [f]: ß-glucosidase enzyme activity; [g]: Aryl sulphatase enzyme activity; [h]: Urease enzyme activity; [i]: Half of irrigation waters (50%TWW+50%FW); [j]: Treated wastewater; [k]: Fresh water; [l]: First soil sampling time; [m]: Second soil sampling time. *Means which are an average of three samples and based on dry weight, followed by same regular small letters in the same column (different treatments) and same italic capital letters in the same row (different sampling time) do not differ by adjusted Duncan test ($P<0.05$)

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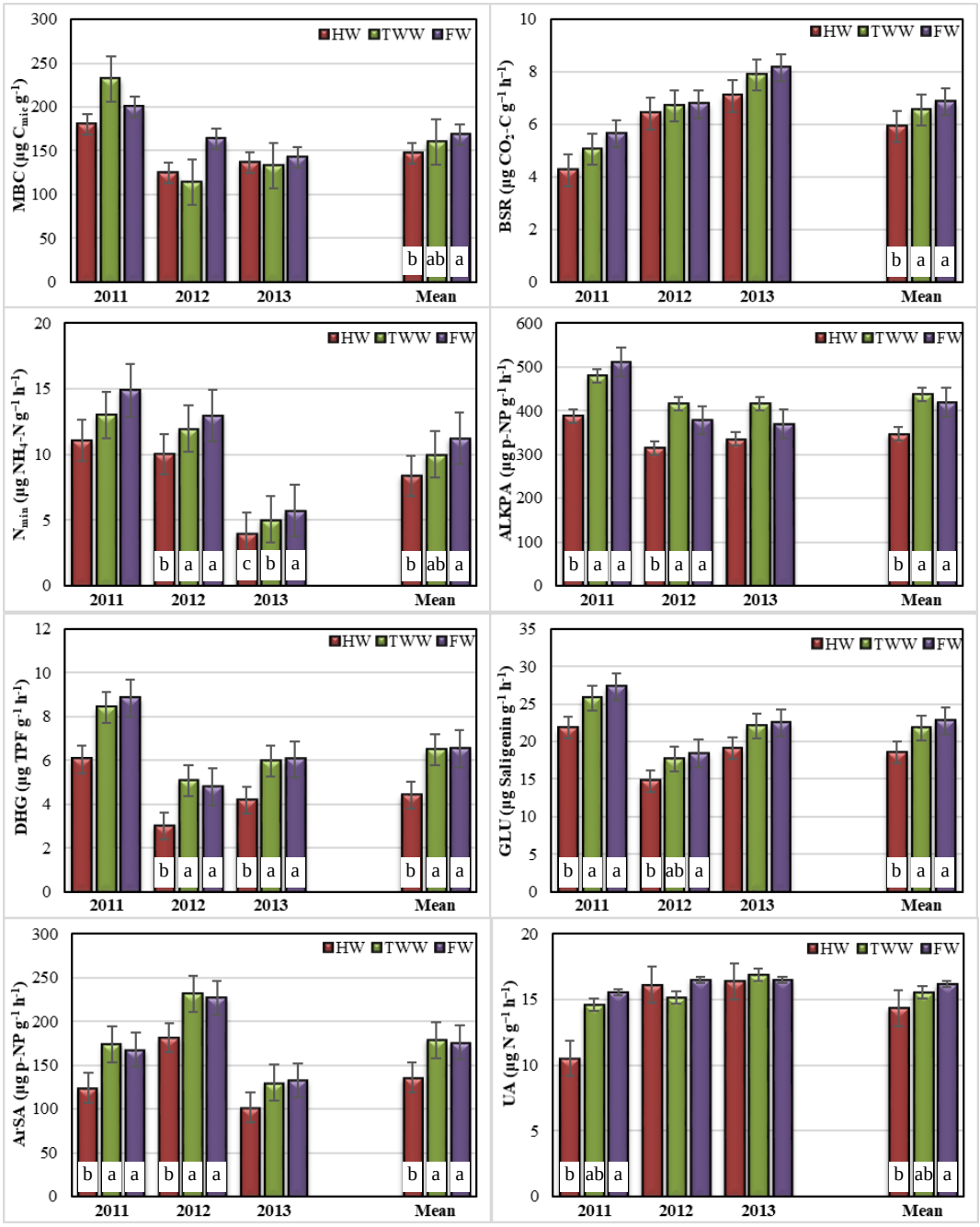

Figure 3. *The effect of different irrigation water quality on microbial biomass carbon content (MBC), basal soil respiration value (BSR), N-mineralization (N_{min}) and the activities of alkaline phosphatase (ALKPA), dehydrogenase (DHG), ß-glucosidase (GLU), aryl sulphatase (ArSA) and urease (UA) of experimental soils. Each value with error bars is the mean of six replicates of two sampling times (S_1 and S_2) in each individual years. The different lowercase letters in each figure do not same by adjusted Duncan test ($P<0.05$)*

It was determined that this may derive from the organic carbon in the wastewater, which supports a simultaneous increase in DHG activity, which is a parameter showing the biological oxidation of organic compounds (Elifantz et al., 2011, Alguacil et al.,

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2012; Frenk et al., 2014). In contrast to this, it was possible in our study to reduce by 90% the organic material in the wastewater by using biological processes in a secondary purification (Pescod, 1992; Veenstra et al., 1997; US-EPA, 2012), so that wastewater was used which is thought not to have had a sufficient organic load, and a reduction was seen in MBC value of 5% when TWW was applied and of 13% when HW was used in comparison with when FW was applied. Similarly, it was found that applications of wastewater treated secondarily (BOD_5: 13.5 mg O_2 L^{-1}) and advanced (BOD_5: 5.8 mg O_2 L^{-1}), which were stated to be very low in organic matter in comparison with other wastewater values, only supplied certain nutrients to the soil, which only slightly stimulated the biomass (Morugán-Coronado et al., 2011). In a study which constituted an example of long-term irrigation with wastewater, drip irrigation with TWW and FW was monitored for 45 years, and it was shown that the type of water did not affect the MBC value (García-Orenes et al., 2015). Evaluating MBC activity on a period basis showed an extreme reduction in period S_2 compared with period S_1 in all years. Along with the harvest period, it may have caused a reduction in the general microbial population under the influence of climatic factors. The highest correlation with MBC, which can be expressed as the C value of the total microbial population was shown by DHG, which can be stated as the oxidative capacity of the total microbial population (r=0.817**, *Table 4*). A high correlation has been widely reported between DHG and MBC (García-Gil et al., 2000, Taylor et al., 2002; Adrover et al., 2012). In addition to this, it has shown a positive correlation with ALKPA, GLU, N_{min} and yield values. The only parameter affecting the MBC value negatively is total Zn (r= -0.467**). Therefore, it can be said that the regular application over three years during the vegetation period of maize plants with a total of 1620 mm of secondary treated wastewater did not result in a large decrease (-5%) in the general microbial population in agricultural soil due to its physicochemical characteristics.

Table 4. *Correlation between average values of some chemical and microbiological parameters of maize soils regardless of irrigation water type at the end of the 3-year experiment*

Parameters[a]	MBC	BSR	ALKPA	DHG	GLU	ArSA	UA	N_{min}
MBC[b]	1	-0.572**	0.643**	0.817**	0.650**			0.380**
BSR[c]	-0.572**	1		-.0600**				-0.610**
ALKPA[d]	0.643**		1	0.755**	0.798**	0.332*	-0.286*	0.458**
DHG[e]	0.817**	-0.600**	0.755**	1	0.762**			0.480**
GLU[f]	0.650**		0.798**	0.762**	1			0.290*
ArSA[g]			0.332*			1		0.491**
UA[h]			-0.286*				1	
N_{min}[i]	0.380**	-0.610**	0.458**	0.480**	0.290*	0.491**		1
Yield[j]	0.313*	-0.408**	0.518**	0.476**	0.624**			0.439**
Total Cr		0.380**				-0.659**		-0.737**
Total Cd							0.362**	
Total Co	0.401**					0.457**		0.292*
Total Ni								
Total Pb						0.756**		0.585**
Total Cu				0.398**		-0.341*		-0.295*
Total Zn	-0.467**	0.458**	-0.556**	-0.474**	-0.563**		0.362**	-0.508**

[a]: The units of each variables are those given in Table 3; [b]: Microbial biomass carbon; [c]: Basal soil respiration; [d]: Alkaline phosphatase enzyme activity; [e]: Dehydrogenase enzyme activity; [f]: ß – glucosidase enzyme activity; [g]: Aryl sulphatase enzyme activity; [h]: Urease enzyme activity; [i]: N-mineralization; [j]: Maize grain yield.

**: Correlation is significant at the 0.01 level; *: Correlation is significant at the 0.05 level.

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Basal soil respiration (BSR)

One of the basic indicators of the microbiological quality of the soil is soil respiration. This is defined as production or consumption of oxygen by the bacteria, fungi, algae and protozoa in the soil. Soil respiration takes place as a result of the breakdown of organic materials, and CO_2 gas is the final product of C-mineralization. Measurement of the amount of CO_2 coming from soil provides important information on both the total biological activity of the soil and the organic material quality of the soil. According to the variance analysis table, although independent variables by themselves have an effect at a level of 1% on BSR, the effect of interactions has not been determined at a significant level (*Supp. Table 2*). With the commencement of crop production and agricultural operations on experimental land which had not been worked for two years, an increase was seen in the BSR value over three years (*Fig. 3*). On the other hand, differences relating to the use of different irrigation waters between mean values of the amount of BSR found in soil samples taken at regular intervals over three years were not found to be significant (5.92 – 6.85 μg CO_2-C g^{-1} h^{-1}; P>0.05). Similarly, in a study conducted in Spain irrigation with TWW over a period of approximately 20 years showed a mean BSR value from 21 different soils of 5.57 μg CO_2-C g^{-1} h^{-1}, and this value was found not to be significantly different from that of soils not irrigated with TWW (4.99 μg CO_2-C g^{-1} h^{-1}) (Adrover et al., 2012). Also similarly, in a study which obtained BSR values in a range of approximately 0.84-3.96 μg CO_2-C g^{-1} h^{-1}, no significant difference was found between FW and TWW (Elifantz et al., 2011). Similar to these results, in this study, FW and TWW applications were also grouped in a similar as per three-year mean value. This result shows us that the load of organic material of the secondarily purified wastewater used in the experiment was not of an amount to create a difference. It may be thought that the fact that the BSR values determined in the soil samples taken at harvest time (SP_2) were the same as or higher than those of SP_1 soils (*Table 3*) may result from (a) salts deriving from TWW and preventing chemoheterotrophic microbial activity being washed down below the rhizosphere zone because of irrigations, and (b) organic material accumulating in the soil, coming from vegetative production in the soil at the end of the period of vegetation of the maize plants. Of course, BSR values may be raised by rhizosphere products such as root secretions and dead cells, C input from plant roots and C from easily decomposed plant product wastes. In addition, it must not be ignored in the explanation of the gap between sampling periods that soil temperature plays a bigger role than soil moisture (Kosugi et al., 2007). Further, application of the same TWW to soils under different plant vegetation can result in big differences in BSR values (Bastida et al., 2018). Also, different BSR values exist which were obtained with TWW applied to soils of different types and thus different chemical characteristics from the structure of the Typic Xerofluvent soil used in this study. An increase was seen in BSR value in Vertic Xerocrept soils irrigated with TWW (Meli et al. 2002), and a reduction was determined in Eutric Histosol soils (Brzezinska et al., 2006). Another interesting result from the study was the negative correlation between BSR and MBC (r = -0.572**, *Table 4*). Finding a negative correlation between these two, where a positive correlation is more generally seen, may be an indicator that the size of the autochthonous flora such as chemoorganotrophs within the general microbial population may not be large, and also that indigenous flora such as chemolithotrophs and photolithotrophs were more stimulated by the applications (Becerra-Castro et al., 2015). The negative correlation determined between BSR and MBC was confirmed by a negative correlation (r = -

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0.600**) also being shown between DHG activity, which showed a positive correlation with MBC, and BSR.

Nitrogen mineralization (N_{min})

N-mineralization, the conversion of nitrogen in the organic form to the inorganic form, is performed by microorganisms with different physiological characteristics. The ammonium and nitrate compounds which result from the two stages in this process, ammonification and nitrification, are basic ions in the supply of nitrogen to plants. In our study, ammonium ions were detected, which were the result of ammonification. The esults of variance analysis showed a significant effect of different irrigation water applications, years and sampling time on N-mineralization in the soil ($P<0.01$). Alongside this, the interaction of the parameter year x sampling period (Year x SP) was found to be significant at the 1% level (*Supp. Table 2*). The high N_{min} value determined in the soil samples taken in the first period after the experiment was set up later returned to a stable position and differences between SPs were not found at a significant level. Examining the mean values of the amounts of N-mineralization determined in six different periods during the experiment (8.38 - 11.21 μg NH_4-N g^{-1} h^{-1}), it was seen that the highest amount of N_{min} was with the FW application (*Fig. 3*). With FW application, N_{min} values were analyzed as 11% higher than with TWW, and 25% higher than with HW. Although N_{min} values relating to TWW applications were lower than with FW applications, we found that this difference was not significant ($P>0.05$). This finding is similar to a study by Roohi et al. (2017).

In other study by Elifantz et al. (2011), which determined that concentrations of organic N and NH_4-N were the main components of wastewater affecting soil quality and biological activity, it was observed that the concentration of NH_4-N in soils to which TWW had been applied for four years did not change, but that the concentration of NO_3-N increased. Accordingly, in this study also, even though the TWW irrigation water used contained a higher level of NH_4-N (0.899 mg L^{-1}) than the FW (*Table 2*), its lower N_{min} value (*Table 3*) suggests that the ammonium contained in the TWW was turned into NO_3 by nitrification carried out by biooxidation by the microorganisms in the autochthonous flora of the soil (Master et al., 2004; Elifantz et al., 2011). Hassen et al. (1998) found that in parallel with the increase in organic carbon in the soils, N-consumption (immobilization) increased in the first month due to adaptation of the microbial population to the new conditions, and that later on N-mineralization and microbial activity increased. Similarly, in our study, a reduction in N_{min} values was accompanied from the first year by an increase in BSR values. This also confirms the negative correlation at a level of r = -0.610** between BSR and N_{min}. A correlation was found between the N_{min} value and the biological parameters analyzed in the study except for UA, and positive correlations with them except for BSR (*Table 4*). The totals of the heavy metals Cr, Cu and Zn affected nitrogen mineralization negatively, while a positive correlation was seen with Co and Pb. In this study, the highest negative correlation with heavy metals was found between N_{min} and total Cr (r = -0.737**).

Evaluation in relation to biochemical parameters

Alkaline phosphatase enzyme (ALKPA) activity

Phosphatases, which are produced by nearly all microorganisms, are enzymes which have agronomic importance in hydrolyzing organic phosphorus compounds and turning

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them into forms which can be taken up by plants (Amador et al., 1997). The individual effects of the independent variables on ALKPA activity in soils was found to be significant at a level of 1%, and only with the interaction Year x Treatment was the effect significant at a level of 5% (*Supp. Table 2*). Mean values of ALKPA activity were found to be in the range of 346.36 to 437.27 μg p-NP g^{-1} h^{-1}, and similar to studies by Adrover et al. (2017) and Bastida et al. (2018), no significant difference was found between TWW and FW applications (P>0.05) (*Fig. 3*). However, in a study conducted in Spain, in which irrigation with TWW was performed for approximately 20 years, an increase at a level of 130% in ALKPA activity was recorded in the area irrigated with TWW (Adrover et al., 2012). Similarly, Brzezinska et al. (2006) and Truu et al. (2009) reported a significant increase in ALKPA activity in soils irrigated with TWW over shorter periods of four and three years respectively. Seasonal variation affected ALKPA activity, and ALKPA activity showed a reduction in all SP_2 samples (P<0.05, *Table 3*). It may therefore be said that conditions of low humidity and heat limit ALKPA activity. Of the heavy metals, only total Zn showed a negative correlation with ALKPA (r = -0.556**, *Table 4*).

Dehydrogenase enzyme (DHG) activity

Dehydrogenases are an intracellular enzyme functioning within living cells. Different from other enzymes, which have been observed functioning both within and outside the cell, DHG gives more reliable information on the size and activity of the living microbial population (Bergstrom et al., 1998). Year, sampling period, treatments and year x SP interaction were found to be significant on DHG activity at a level of P<0.01, and SP x Treatment and Year x SP x Treatment interactions at a level of P<0.05 (*Supp. Table 2*). The mean values of amounts of DHG found in soil samples taken at regular intervals over a period of 36 months were found to range between 4.40 and 6.54 μg TPF g^{-1} h^{-1} (*Fig. 3*). The FW and TWW applications showed similar DHG activity at the end of the three-year experimental period, while in the HW application, DHG activity showed a 17% reduction. Similarly, no significant difference was found between DHG activity on soil of which TWW was or was not applied to the soil under Mediterranean climatic conditions over a period of approximately 20 years (Adrover et al., 2012). Along with this, similar DHG activity determined in the soil in connection to different irrigation waters may be related to the low local organic C content of the soil (Bastida et al., 2006) and also to the organic matter load of the TWW used, as has been reported from soil used in this study and dry regions. In contrast to these findings, DHG activity in other studies was generally higher on soils irrigated with TWW or untreated WW than on soil irrigated with fresh water (Gianfreda et al., 2005; Chen et al., 2008). An increase in soil DHG activity also derives from the microbial use of nutrients which can be easily obtained from wastewater as a source of C, N and energy (Arif et al., 2016). Positive effects have previously been reported of wastewater containing highly unstable organic C on soil enzyme activities (Marín-Benito et al., 2012; Liang et al., 2014). A decrease in DHG activity at a significant level was found in relation to seasonal changes in SP_2 sampling periods compared to SP_1 sampling periods (*Table 3*). However, in a study conducted over four years by Elifantz et al. (2011), it was reported that there was no effect on DHG activity of a seasonal change coinciding with different sampling periods. Nevertheless, because dehydrogenase enzyme activity is connected to the total activity of the living microbial population, the amount in soils varies according to the microbial population level (Skujins, 1976), and in this study also a strong positive correlation was found between MBC and DHG (r = 0.817**, *Table 4*). A similarly

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strong correlation was found by Adrover et al. (2012) ($r = 0.735$, $P<0.001$). For this reason, abiotic factors affecting the microbial population may be expected to affect DHG activity. In addition to this, a positive correlation was found at a level of r = 0.398**between DHG activity and total Cu, while a negative correlation at a level of r = -0.474**was found with total Zn.

ß-glucosidase enzyme (GLU) activity

Low molecular weight sugars, which are a product of glucosidase hydrolysis, are an important source of energy for soil microorganisms. One of the most important glucosidases in the soil is β-glucosidase, which contributes to the mineralization of cellulose, the principal organic carbon compound in nature (Landgraf, 2003). GLU is a widely-used parameter in the evaluation of soil quality and biochemical functionality after using different types of soil management (Bandick and Dick, 1999). It was found that the effect of the independent variables by themselves on GLU activity was significant at the 1% level, but their interactions were found not to be significant (*Supp. Table 2*). The mean values of the amount of GLU activity were found to be between 18.55 and 22.74 μg Saligenin g^{-1} h^{-1} (*Fig. 3*), and it was seen that the highest GLU activity was with the FW application. In a study conducted by Bastida et al. (2018) in Spain under Mediterranean climatic conditions, a similar amount of FW and TWW to that in our study but with 67% and 118% more salinity respectively and using a drip irrigation system was applied to grapefruit and mandarin plantations. The researchers found more GLU activity under grapefruit irrigated with TWW than with FW, while under mandarins no significant difference was found in GLU activity ($P>0.05$). Similarly, the effect on GLU of FW and TWW applications in this study was found not to be significant ($P>0.05$), while the differences with applications of HW were found to be significant ($P<0.05$). The direction and severity of the difference appearing in applications of TWW were closely related to the existence of a local resistant and adaptable microbial community which is the source of extracellular enzymes such as GLU and can therefore maintain the nutrient cycle in the soil (Adrover et al., 2012; Bastida et al., 2018). Also, as a result of long-term TWW application, β-glucosidase enzyme activity, which is part of the C cycle, showed an increase in relation to the amount of C entering the soil (Adrover et al., 2012). The entry of C to the soil is not only connected to the application of wastewater, but at the same time can occur in relation to the kind of crop produced (Dodor and Tabatabai, 2005). The highest GLU activity values obtained by these authors were found in soils just under an alfalfa crop. Similarly, Truu et al. (2009) showed the joint effect of municipal wastewater and crop cover on the soil microbial community. Hydrolytic activity potential in the soil can show distinct seasonal patterns (Elifantz et al., 2011). Therefore, in a similar way to ALKPA, an enzyme of the hydrolase group, GLU activity showed a significant reduction in SP_2 samples, a period in which soil humidity is not high (*Table 3*). While seasonal change affects biochemical activity of microbial origin, it basically affects the microbial population which exhibits this biochemical activity in the background. As both Turner et al. (2002) and Adrover et al. (2012) found, the high correlation between GLU and MBC ($r = 0.650$**, *Table 4*) is because the activity of this enzyme is basically related to the microbial biomass in the soils (Dodor and Tabatai, 2005). A negative correlation at the level of r = -0.474** was found between GLU activity and total Zn, but no relation was shown with other heavy metals.

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Aryl sulphatase enzyme (ArSA) activity

ArSA is an enzyme responsible for the fusion of oxygen-sulphur bonds and the hydrolysis of aryl sulphatase esters (Spencer, 1958). In other words, it is thought to be involved in the mineralization of ester sulphate in the soil (Tabatabai, 1994). Because ester sulphate, the substrate of ArSA, is only found in fungi, this enzyme can also be an indirect indicator of fungi in the soil (Bandick and Dick, 1999). According to the variance analysis table, a significant effect on ArSA activity of the year, sampling period, treatments, and of the interactions only year x sampling period, was found at the 1% level (*Supp. Table 2*). ArSA activity was determined at about the same level in the TWW and FW applications, and this may be an indicator that a useful substrate of the enzyme is not found at high levels in wastewater. Furthermore, the absence of a reduction in ArSA activity in relation to the application of TWW, and even in increase at a level of 2% relative to FW, may be a sign that the composition of the TWW did not have a drastically negative effect on fungal activity in the soil. The application of HW caused ArSA activity behind that of the other two applications (*Fig. 3*). Kayikcioglu (2012) reported a reduction in ArSA activity in agricultural soils when municipal wastewater was used for irrigation. However, an increase in ArSA activity was observed over a period of more than 10 years by Chen et al. (2008) and over a period of more than 20 years by Adrover et al. (2017). Parameters that are primarily effective on these difference of increase and decrease in ArSA activity are the composition of wastewater and its concentrations, irrigation duration, the characteristics of the irrigation water and irrigated soil, which at the same time decide on the final effects on the functional properties of the microorganisms in the wastewater irrigated soils (Elifantz et al., 2011; Lopes et al., 2015). Comparing periods, a greater level of ArSA activity was obtained in S_1 samples than in S_2 samples for all three applications; these differences were found to be significant ($P<0.05$) in the first two years only with the TWW application, but in the last year in all applications (*Table 3*). It can therefore be said that the ArSA is not successful in adsorbing the colloidal fraction in the soils, and that its amount is reduced by such factors as thermal denaturation, dehydration and protolysis. Examining the correlation matrix, it is seen that the highest correlation was interestingly with total Pb ($r = 0.756^{**}$, *Table 4*). Also surprisingly, a significant correlation at the 1% level was determined with total Co. On the other hand, negative correlations were determined between ArSA activity and total Cr (-0.659**) and total Cu (-0.341**).

Urease enzyme (UA) activity

Urease is an enzyme which catalyzes the hydrolysis of urea to ammoniac or ammonium, depending on soil pH (Tripathi et al., 2007). According to the results of variance analysis, the applications were found to be statistically insignificant only on UA activity. On the other hand, the effect of year and sampling period, and year x SP interaction was found to be significant at the level of $P<0.01$, while year x treatment and SP x treatment interactions showed a significant effect at the $P<0.05$ level (*Supp. Table 2*). Even though the mean UA activity values obtained in the experiment were analyzed as being very close to each other (14.34 – 16.6 $\mu g\ N\ g^{-1}\ h^{-1}$), the differences were found to be significant ($P<0.05$) (*Fig. 3*). The highest value was obtained with the FW application, and this was followed first by the TWW application and then by the HW application. A previous study showed that UA enzyme activity was hindered by an

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increase in heavy metal pollution in soils which had been irrigated with wastewater for long periods (Hu et al., 2014). In contrast, there are studies in which UA activity obtained when TWW was applied to soils under grapefruit and mandarin plantations was higher than in soils to which FW had been applied (Bastida et al., 2018). Furthermore, while long-term (45 year) application of wastewater was found to cause a significant increase in UA, GLU and ALKPA activities, this increase was found to be greatest (41%) in UA and GLU activities in comparison with that in soils irrigated with FW (García-Orenes et al., 2015). In years other than the first year, UA activity was analyzed at a more clearly higher level in SP_2 samples (Table 3). The highest UA activities were found in third-year SP_2 samples, corresponding to the sixth period. Yang et al. (2006) observed that catalase, invertase, urease and alkaline phosphatase activities were significantly decreased by an increase of Cd in the soil. In contrast to this result, a correlation was found in this study at a level of r = 0.362** between UA activity and total Cd.

Evaluation relating to grain yield of maize

The effect of the different irrigation water applications in the study on the grain yield of the experimental plant, maize, were investigated, and the results are shown in *Fig. 4*.

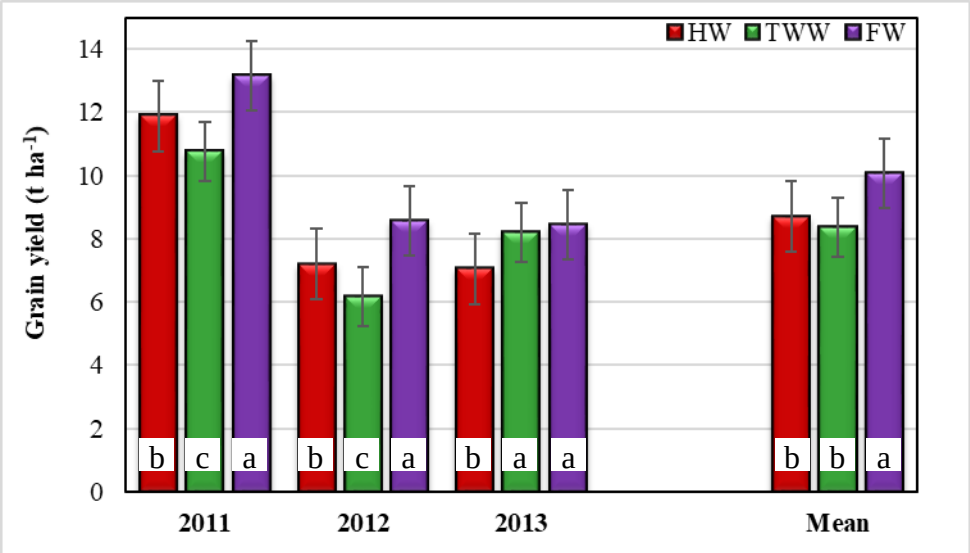

Figure 4. *The effect of different irrigation water quality on grain yield of maize. Each year's value with error bar is the mean of three replicates in each individual years. The different lower case letters in each figure do not same by adjusted Duncan test (P<0.05)*

Taking into account three-year means, it was found that yields were lower by 17% with the application of 100% secondarily treated wastewater (TWW) and by 13% with a half-and-half dose (HW) in comparison with the application of FW, and that these differences were significant (P<0.05). However, although lower crop yields were seen with the application of TWW in some other studies, these did not reach the level of statistical significance. For example, in a study in Spain with two different salt-sensitive fruit trees, no difference at a significant level was found in terms of fruit yield between applications of TWW and FW (Bastida et al., 2018). Besides similarly, irrigation of a

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persimmon orchard with TWW for four years with TWW, fruit yield was not affected in comparison with trees irrigated with fresh water (Elifantz et al., 2011). Maize yield was found to be 1005.67 kg da^{-1} with FW application. In another study, it was possible to achieve a reduction in the amount of fertilizer given thanks to the nutrients in the TWW and at the same time to achieve the same olive production by means of traditional techniques (Segal et al. 2011; Bedbabis et al., 2015). A positive correlation was found between yield parameters and the biological parameters of the N, P and C cycles and the general microbial population. Similar to this result, positive correlations was determined in a 6-year study using dried olive mill wastewater between fig yield and BSR, UA and ALKPA as 0.298, 0.251 and 0.421 ($P<0.01$), respectively (Kayikcioglu and Sahin, 2013). Kayikcioglu and Sahin (2013) also suggested that dried olive mill wastewater applications at a rates of 75 kg $tree^{-1}$ for every year and 100 kg $tree^{-1}$ for every two years are the best amendment rates for degraded and poor Mediterranean soils and fig orchards in the arid and semi arid region.

Principal Component Analysis – PCA

PCA analysis was performed to measure the effects and interactions of the applications on soil biological variables. The first step in factor analysis was to test the suitability of the data, and the Kaiser-Meyer-Olkin (KMO) coefficient and the Bartlett Sphericity test were used for this (Tabachnick and Fidell, 2014). Because the KMO value was 0.615>0.500 and the Bartlett value was $P<0.05$ in the PCA analysis of the microbiological parameters analysed in this study, these values were found to be significant and showed that the constituents of the data set were suitable at a medium level in the factor analysis (Kaiser and Rice, 1960; Sharma, 1996). The second step in factor analysis was to investigate all variables with principal component analysis. Factors with an eigenvalue statistic of more than 1 were taken as significant, while those, which are smaller, were disregarded (Kaiser and Rice, 1960). These variables were evaluated by the Oblimin rotation method (Rotation Method: Oblimin with Kaiser Normalization), and a three-factor model was obtained as the most suitable (*Fig. 5; Supp. Table 3; Supp. Fig. 1*).

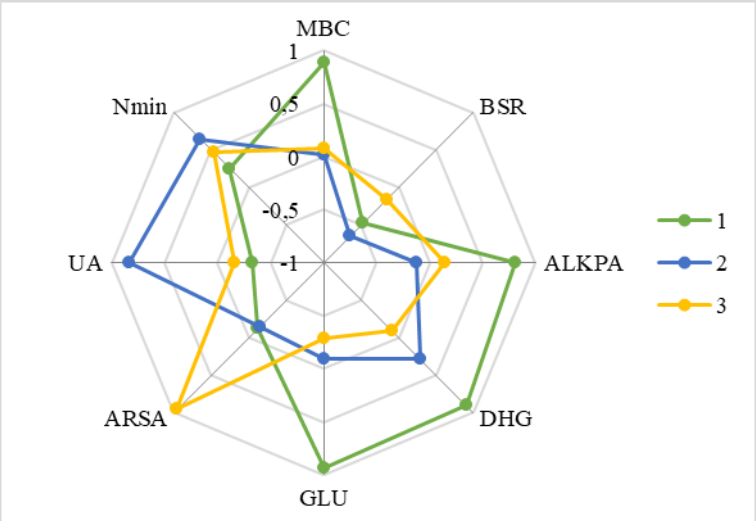

Figure 5. *Plot of three main principle factors (1, 2 and 3) based on PCA for the irrigation water type and the first, second and third principal components of soil microbial properties*

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A structure of three factors and eight variables was determined as a result of the factor analysis to explain the effect of the application of irrigation water with different characteristics on the components of soil health. Finally, this structure of three factors and eight variables explained 83.1% of the variance. GLU was the factor, which most affected total factor formation by 96%. The correlation values between factors were found to be 0.090 between Factor 1 and Factor 2, 0.261 between Factor 1 and Factor 3, and 0.027 between Factor 2 and Factor 3, and because all factors were below 30%, it can be said that the separation between factors was good (*Supp. Table 5*). The first factor (eigenvalue 3.91) can explain 48.90% of the total variance, the second factor (eigenvalue 1.41) can explain 17.56%, and the third factor (eigenvalue 1.33) can explain 16.60% (*Supp. Table 3; Supp. Fig. 1*). Examining the variables in the factors according to the Pattern Matrix, MBC, DHG, GLU, and ALKPA have contributed significantly in Factor 1, UA, N_{min} and BSR in Factor 2, and ArSA in Factor 3 (*Fig. 5; Supp. Table 4*). Therefore, the contribution, which they made to the soil functions, was taken into account in naming them. These three factors were named respectively C-P-Cycles and Microbial Oxidation Capacity, N-Cycle, and S-Cycle.

PCA analysis was performed for the chemical characteristics (organic carbon, heavy metals, pH, etc.) of the soils included in the study, and because the KMO value showing the suitability of the components was found to be 0.516, analysis could not be continued. If it had reached validity, 76.38% of total variance could have been explained by four factors as a result of PCA. Nevertheless, the variance, which could explain the chemical parameters with a large number of components, would have been left behind that of the microbiological parameters. Therefore, any agricultural management practices carried out on the soil, in order for it to be evaluated with regard to the soil ecosystem, definitely needs the microbiological parameters of the soil, which respond fastest and most correctly.

Conclusion

Research on the potential for using treated wastewater in agriculture without compromising on concerns for food safety has gained great importance, particularly in areas with an arid or semi-arid climate. This is because of unprecedented pressure on renewable but limited water resources from population increase and demographic growth. Therefore, the use of wastewater in irrigation is seen as a way of righting the imbalance between water demand and supply. However, some studies of wastewater have indicated adverse effects on the soil or on crop characteristics. These will affect soil fertility and productivity and will increase concerns about the sustainability of the re-use of treated wastewater in agriculture. A cumulative impact and evaluation mechanism that is necessary in order to determine potential for use of treated wastewater as an alternative source of irrigation water, will be emerged when the TWW effect especially on microbiological and biochemical activity, which are important parameters in fulfilling ecosystem functions of the soil, is also supported by its effect on plant productivity. In line with this aim, in this study secondarily treated municipal wastewater, fresh water, and a 1:1 mixture of these two were applied to Typic Xerofluvent soil in Western Anatolia, under Mediterranean climatic conditions, and maize was grown as a test crop. In the results of the three-year field experiment, it was observed that the effect of irrigation with treated wastewater and fresh water on biological activity were very close to one another. The difference was not significant

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(P>0.05), with an average reduction of 3%. Analysis of soil samples taken in six periods over a total of three years showed that seasonal variation had a significant effect on certain dependent variables. In comparison with SP_1 soil samples, MBC, ALKPA, DHG and GLU parameters were analyzed at lower levels in SP_2 soil samples, while BSR, N_{min}, ArSA and UA activities were found to be the same or at a higher level (P<0.05). Another result obtained from the study was the level of correlation between heavy metals and the biological parameters analyzed. A positive relationship was found in the study between the biological parameters and total Cd, Co and Pb, while both positive and negative correlations were found with total Cr, Cu and Zn. The only element which showed no correlation was total nickel. Another parameter observed in the experiment was the productivity of the maize plants. A negative effect of the application of wastewater emerged from the yield parameter (P<0.05). Irrigation with diluted treated wastewater (HW) and 100% treated wastewater (TWW) created a reduction in yield of 13% and 17% respectively. However, it is appropriate to calculate the possible loss in crop yield according to the economic realities of local conditions, and to decide on the application of TWW in this way. In any case, taking account of current studies in choosing crops to grow with treated wastewater may reduce yield losses to a minimum. In this way, in arid or semi-arid regions where there is a lack of rainfall and a problem with water resources, and where there is seawater intrusion into the aquifers, it can be seen as providing a good alternative. However, in order to re-use wastewater in a sustainable way, parameters affecting both soil health and food safety must be included in quality standards.

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APPENDIX

Supplementary Table 1. *Multivariate hypothesis tests (MANOVA)[a] in terms of independent variables*

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	0.998	1884.704[b]	8.000	27.000	0.000
	Wilks' Lambda	0.002	1884.704[b]	8.000	27.000	0.000
	Hotelling's Trace	558.431	1884.704[b]	8.000	27.000	0.000
	Roy's Largest Root	558.431	1884.704[b]	8.000	27.000	0.000
Year	Pillai's Trace	1.884	56.887	16.000	56.000	0.000
	Wilks' Lambda	0.003	57.162[b]	16.000	54.000	0.000
	Hotelling's Trace	35.295	57.354	16.000	52.000	0.000
	Roy's Largest Root	22.746	79.610[c]	8.000	28.000	0.000
Sampling Period	Pillai's Trace	0.968	100.747[b]	8.000	27.000	0.000
	Wilks' Lambda	0.032	100.747[b]	8.000	27.000	0.000
	Hotelling's Trace	29.851	100.747[b]	8.000	27.000	0.000
	Roy's Largest Root	29.851	100.747[b]	8.000	27.000	0.000
Treatment	Pillai's Trace	1.220	5.479	16.000	56.000	0.000
	Wilks' Lambda	0.098	7.392[b]	16.000	54.000	0.000
	Hotelling's Trace	5.934	9.643	16.000	52.000	0.000
	Roy's Largest Root	5.325	18.638[c]	8.000	28.000	0.000
Replication	Pillai's Trace	0.520	1.230	16.000	56.000	0.275
	Wilks' Lambda	0.540	1.218[b]	16.000	54.000	0.285
	Hotelling's Trace	0.741	1.204	16.000	52.000	0.296
	Roy's Largest Root	0.532	1.863[c]	8.000	28.000	0.107
Year * Sampling Period	Pillai's Trace	1.728	22.232	16.000	56.000	0.000
	Wilks' Lambda	0.011	28.110[b]	16.000	54.000	0.000
	Hotelling's Trace	21.675	35.221	16.000	52.000	0.000
	Roy's Largest Root	18.124	63.433[c]	8.000	28.000	0.000
Year * Treatment	Pillai's Trace	1.108	1.437	32.000	120.000	0.083
	Wilks' Lambda	0.244	1.472	32.000	101.166	0.076
	Hotelling's Trace	1.862	1.484	32.000	102.000	0.071
	Roy's Largest Root	1.042	3.908[c]	8.000	30.000	0.003
Sampling Period * Treatment	Pillai's Trace	0.744	2.071	16.000	56.000	0.023
	Wilks' Lambda	0.379	2.107[b]	16.000	54.000	0.022
	Hotelling's Trace	1.315	2.138	16.000	52.000	0.020
	Roy's Largest Root	0.988	3.459[c]	8.000	28.000	0.007
Year * Sampling Period * Treatment	Pillai's Trace	1.193	1.593	32.000	120.000	0.038
	Wilks' Lambda	0.191	1.790	32.000	101.166	0.015
	Hotelling's Trace	2.436	1.941	32.000	102.000	0.007
	Roy's Largest Root	1.415	5.306[c]	8.000	30.000	0.000

a. Design: Intercept + Year + Sampling Period + Treatment + Replication + Year * Sampling Period + Year * Treatment + Sampling Period * Treatment + Year * Sampling Period * Treatment
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Supplementary Table 2. *Multivariate hypothesis tests (MANOVA) that shows the interaction between dependent and independent variables*

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	MBC	471020.089[a]	19	24790.531	38.696	0.000
	BSR	183.718[b]	19	9.669	12.737	0.000
	ALKPA	280884.414[c]	19	14783.390	11.174	0.000
	DHG	430.160[d]	19	22.640	36.548	0.000
	GLU	1035.678[e]	19	54.509	18.221	0.000
	ARSA	115802.378[f]	19	6094.862	17.786	0.000
	UA	1696.251[g]	19	89.276	17.903	0.000
	NMIN	937.055[h]	19	49.319	21.863	0.000

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Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	MBC	1354255.764	1	1354255.764	2113.868	0.000
	BSR	2239.059	1	2239.059	2949.382	0.000
	ALKPA	8686501.602	1	8686501.602	6565.867	0.000
	DHG	1822.783	1	1822.783	2942.570	0.000
	GLU	23920.505	1	23920.505	7995.953	0.000
	ARSA	1440069.542	1	1440069.542	4202.324	0.000
	UA	12716.248	1	12716.248	2550.007	0.000
	NMIN	5255.303	1	5255.303	2329.691	0.000
Year	MBC	55542.767	2	27771.383	43.349	0.000
	BSR	67.836	2	33.918	44.678	0.000
	ALKPA	92629.276	2	46314.638	35.008	0.000
	DHG	112.991	2	56.495	91.202	0.000
	GLU	578.632	2	289.316	96.710	0.000
	ARSA	77709.663	2	38854.832	113.384	0.000
	UA	91.015	2	45.507	9.126	0.001
	NMIN	668.672	2	334.336	148.212	0.000
Sampling Period	MBC	353145.866	1	353145.866	551.228	0.000
	BSR	20.456	1	20.456	26.946	0.000
	ALKPA	75383.290	1	75383.290	56.980	0.000
	DHG	133.658	1	133.658	215.768	0.000
	GLU	220.734	1	220.734	73.785	0.000
	ARSA	9560.255	1	9560.255	27.898	0.000
	UA	370.227	1	370.227	74.242	0.000
	NMIN	18.506	1	18.506	8.204	0.007
Treatment	MBC	4130.921	2	2065.460	3.224	0.052
	BSR	8.117	2	4.058	5.346	0.010
	ALKPA	83639.539	2	41819.769	31.610	0.000
	DHG	53.460	2	26.730	43.151	0.000
	GLU	174.874	2	87.437	29.228	0.000
	ARSA	20517.476	2	10258.738	29.936	0.000
	UA	31.031	2	15.515	3.111	0.057
	NMIN	72.741	2	36.371	16.123	0.000
Replication	MBC	1332.628	2	666.314	1.040	0.364
	BSR	0.358	2	.179	.236	0.791
	ALKPA	2100.882	2	1050.441	.794	0.460
	DHG	2.832	2	1.416	2.286	0.117
	GLU	20.082	2	10.041	3.356	0.047
	ARSA	444.471	2	222.235	.649	0.529
	UA	2.633	2	1.317	.264	0.770
	NMIN	12.560	2	6.280	2.784	0.076
Year * Sampling Period	MBC	26954.159	2	13477.079	21.036	0.000
	BSR	82.825	2	41.412	54.550	0.000
	ALKPA	4253.427	2	2126.713	1.608	0.215
	DHG	112.078	2	56.039	90.466	0.000
	GLU	1.648	2	.824	.275	0.761
	ARSA	5181.656	2	2590.828	7.560	0.002
	UA	1067.847	2	533.924	107.068	0.000
	NMIN	126.156	2	63.078	27.963	0.000
Year * Treatment	MBC	12619.214	4	3154.804	4.924	0.003
	BSR	1.707	4	.427	.562	0.692
	ALKPA	15840.875	4	3960.219	2.993	0.032
	DHG	2.090	4	.523	.844	0.507
	GLU	7.739	4	1.935	.647	0.633
	ARSA	961.743	4	240.436	.702	0.596
	UA	60.692	4	15.173	3.043	0.030
	NMIN	6.782	4	1.695	.752	0.564
Sampling Period * Treatment	MBC	7030.846	2	3515.423	5.487	0.009
	BSR	0.662	2	.331	.436	0.650
	ALKPA	6224.849	2	3112.424	2.353	0.110
	DHG	5.713	2	2.856	4.611	0.017
	GLU	1.519	2	.759	.254	0.777
	ARSA	1004.317	2	502.159	1.465	0.245

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Source		Type III Sum of Squares	df	Mean Square	F	Sig.
	UA	48.645	2	24.322	4.877	0.014
	NMIN	13.427	2	6.713	2.976	0.064
Year * Sampling Period * Treatment	MBC	10263.688	4	2565.922	4.005	0.009
	BSR	1.758	4	.439	.579	0.680
	ALKPA	812.277	4	203.069	.153	0.960
	DHG	7.339	4	1.835	2.962	0.033
	GLU	30.452	4	7.613	2.545	0.057
	ARSA	422.797	4	105.699	.308	0.870
	UA	24.161	4	6.040	1.211	0.324
	NMIN	18.211	4	4.553	2.018	0.114
Error	MBC	21782.203	34	640.653		
	BSR	25.812	34	.759		
	ALKPA	44981.275	34	1322.979		
	DHG	21.061	34	.619		
	GLU	101.714	34	2.992		
	ARSA	11651.259	34	342.684		
	UA	169.550	34	4.987		
	NMIN	76.697	34	2.256		
Total	MBC	1847058.055	54			
	BSR	2448.589	54			
	ALKPA	9012367.290	54			
	DHG	2274.005	54			
	GLU	25057.896	54			
	ARSA	1567523.179	54			
	UA	14582.048	54			
	NMIN	6269.055	54			
Corrected Total	MBC	492802.291	53			
	BSR	209.530	53			
	ALKPA	325865.688	53			
	DHG	451.222	53			
	GLU	1137.392	53			
	ARSA	127453.637	53			
	UA	1865.800	53			
	NMIN	1013.752	53			

a. R Squared = 0.956 (Adjusted R Squared = 0.931)
b. R Squared = 0.877 (Adjusted R Squared = 0.808)
c. R Squared = 0.862 (Adjusted R Squared = 0.785)
d. R Squared = 0.953 (Adjusted R Squared = 0.927)
e. R Squared = 0.911 (Adjusted R Squared = 0.861)
f. R Squared = 0.909 (Adjusted R Squared = 0.857)
g. R Squared = 0.909 (Adjusted R Squared = 0.858)
h. R Squared = 0.924 (Adjusted R Squared = 0.882)

Supplementary Table 3. *Total variance explained for PCA*

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings[a]
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total
1	3.912	48.900	48.900	3.912	48.900	48.900	3.756
2	1.405	17.557	66.457	1.405	17.557	66.457	1.502
3	1.328	16.595	83.052	1.328	16.595	83.052	1.888
4	0.647	8.086	91.138				
5	0.405	5.068	96.206				
6	0.150	1.874	98.080				

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7	0.103	1.288	99.368
8	0.051	0.632	100.000

Extraction Method was the Principal Component Analysis.
a. When components are correlated, sums of squared loadings cannot be added to obtain a total variance.

Supplementary Table 4. *Pattern matrix of components*[a]

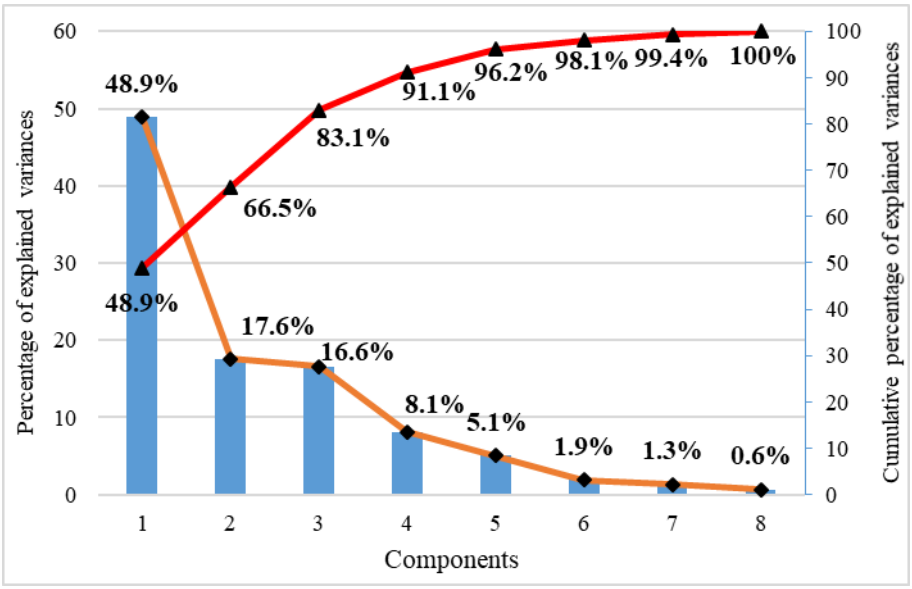
	Component		
	1	**2**	**3**
MBC	**0.847**	-0.034	0.089
BSR	-0.446	**0.679**	-0.151
ALKPA	**0.826**	0.218	0.218
DHG	**0.914**	-0.246	-0.037
GLU	**0.962**	0.163	-0.216
ARSA	-0.151	0.192	**0.966**
UA	-0.287	**-0.806**	-0.144
N_{min}	0.260	**0.692**	-0.404

*Extraction method was the Principal Component Analysis; rotation method was the Oblimin with Kaiser Normalization.
[a]: Rotation converged in 7 iterations.

Supplementary Table 5. *Correlation matrix of components*

Component	**1**	**2**	**3**
1	1.000	0.090	0.261
2	0.090	1.000	0.027
3	0.261	0.027	1.000

*Extraction method was the Principal Component Analysis; rotation method was the Oblimin with Kaiser Normalization.



Supplementary Figure 1. *Variance and cumulative variance explained with the components*

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