

Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health

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Abstract

The reuse of treated wastewater, in particular for irrigation, is an increasingly common practice, encouraged by governments and official entities worldwide. Irrigation with wastewater may have implications at two different levels: alter the physicochemical and microbiological properties of the soil and/or introduce and contribute to the accumulation of chemical and biological contaminants in soil. The first may affect soil productivity and fertility; the second may pose serious risks to the human and environmental health. The sustainable wastewater reuse in agriculture should prevent both types of effects, requiring a holistic and integrated risk assessment. In this article we critically review possible effects of irrigation with treated wastewater, with special emphasis on soil microbiota. The maintenance of a rich and diversified autochthonous soil microbiota and the use of treated wastewater with minimal levels of potential soil contaminants are proposed as *sine qua non* conditions to achieve a sustainable wastewater reuse for irrigation.

1. Reasons behind the use of wastewater for irrigation

Water is essential for life, and although covering approximately 70% of the terrestrial crust area, only a small fraction of the water is actually compatible with terrestrial life forms (Shiklomanov, 1993). The small fraction of freshwater, i.e., with low salt concentration (2.5% of the total stock of water in the hydrosphere), is mainly in the form of ice and permanent snow coating in the Antarctic and Arctic regions (68.7%) (Shiklomanov, 1993). One of the first reasons for the observed water scarcity is that the fraction of water available for the human consumption, in rivers and streams, lakes, reservoirs and groundwater aquifers, is not distributed uniformly around the world (Shiklomanov, 1993). As a consequence, 40% of the total land area is dry and includes climate zones classified as arid, semi-arid and dry sub-humid (FAO, 2008). Simultaneously, the increasing need of water resources is a consequence of the demographic growth, the economic

development and the improvement of living standards, climate change and pollution (FAO, 2012). It is estimated that at least in some world regions, water use has been growing more than twice faster than the human population. For instance, the total annual water withdrawal suffered a 6.3- fold increase, rising from less than 600 km³/year, at the beginning of the twentieth century, to more than 3800 km³/year by the beginning of the twenty-first century. The irrigation practices consume up to 70% of this withdrawal (FAO, 2013). Therefore, the use of freshwater has been exceeding the minimum recharge levels, leading to the desiccation of water streams and depletion of groundwater (UNDP, 2006). Water scarcity and droughts are emerging as major issues worldwide, not only in dry lands, but also in world regions where freshwater is abundant (Bixio et al., 2006; EU, 2007; FAO, 2012). For instance, half of the European countries are facing water stress (Bixio et al., 2006) with water scarcity being observed across Europe (EU, 2007). It is estimated that more than 40% of the world's population will face water stress or scarcity within the next 50 years, a serious incentive to achieve sustainable management options of the water resources (WHO, 2006a). In this context, the reuse of treated wastewater represents a valid option, in some cases urged by the absence of viable alternatives (Niemczynowicz, 1999; WHO, 2006a). Besides the reduction in the use and abstraction of freshwater, wastewater reuse will also contribute to reduce the discharge of effluents into freshwater ecosystems (Bixio et al., 2006; Toze, 2006). This scenario makes wastewater an increasingly valuable resource rather than a waste product. Indeed, irrigation with treated wastewater is already implemented, mainly for agriculture and landscaping, in countries such as France, Italy, Spain, Cyprus, Malta, Israel, Jordan or the USA (Aquarec, 2006; EMWIS, 2007; EPA, 2012; Kalavrouziotis et al., 2013; Ndour et al., 2008; Pedrero et al., 2010). However, despite the aforementioned benefits, the reuse of wastewater involves both health and environmental risks. In this review, we will discuss some implications of the reuse of treated wastewater for irrigation purposes, giving special emphasis to microbiological aspects.

2. Framework of guidelines and policies on wastewater reuse

Wastewater is produced as a result of the multiple human activities, such as domestic, commercial and industrial uses. The quantity and composition of urban wastewater are determined by several factors, including the inhabitant's lifestyle and standard of living, the proportion of domestic and industrial effluents, or even the design of the sewer and of the treatment systems (Henze and Comeau, 2008; Metcalf and Eddy, 2003). Treated urban wastewater is mainly composed by particulate and dissolved organic matter, and inorganic substances (e.g., N, P, K, Na, Ca, Mg, Cl and B), containing also microorganisms, including pathogens and antibiotic resistant bacteria (Henze and Comeau, 2008; Rizzo et al., 2013; Varela and Manaia, 2013). Additionally, toxic, recalcitrant and/or bioaccumulative chemicals (e.g., trace metals, xenobiotics and natural or semi-synthetic compounds) are normally present, although representing minor components, often designated as micropollutants or microcontaminants (Henze and Comeau, 2008; Metcalf and Eddy, 2003). Given such complexity, the detailed chemical and biological characterisation of treated wastewater is essential to assess its quality, being always difficult to fully predict the effects that may arise from its reuse.

The recommendations on wastewater reuse established by the State of California, the World Health Organisation (WHO) and the US Environmental Protection Agency (EPA) (Aquarec, 2006;

EPA, 2012; WHO, 2006a) constitute the background of most of the legal guidelines proposed in countries such as the USA, Portugal, Spain, Italy, Cyprus, France, Australia, Israel, Jordan, Kuwait, Oman, Saudi Arabia, and China (Table 1). Compliance with these regulatory frameworks requires the analysis of the treated wastewater prior to its reuse. Although the reviewed guidelines and policies cover different applications for the wastewater reuse (e.g. irrigation, groundwater recharge, impoundments and industrial reuse) we will focus our discussion on the use of wastewater for irrigation purposes.

In general, standards are based on the evaluation of physicochemical and microbiological parameters. A summary of the main parameters considered in the different guidelines and policies is given in Table 1. Several countries (e.g. the USA, Spain, Jordan) establish different threshold values in function of the type of irrigated crops. For raw-consumed food crops the recommended values are generally stricter than for other crops that will be further processed (processed food) or used as pasture or energy crops. On the other hand, some countries, such as Italy or Israel do not define irrigation categories, and hence the established threshold values should be accomplished regardless the intended use for the irrigated crops (Table 1).

Physicochemical characterisation of wastewater includes the evaluation of several properties such as turbidity [Nephelometric Turbidity Units (NTU) or suspended solids (SS)], acidity (pH), salinity [electrical conductivity (EC), sodium absorption rate (SAR)], organic load [biological (BOD) or chemical oxygen demand (COD)], and nutrients [total N and/or NO_3^- and P in form of PO_4^{3-} phosphate] (Table 1). In addition to these parameters, some regulations (e.g. the USA, Italy, Mexico, Oman) require or recommend the determination of potentially toxic agents such as metals or organic contaminants (Table 1). The microbiological characterisation of wastewater is mainly focused on the presence of potential human pathogens and parasites, and is generally based on the enumeration of faecal indicators and nematode eggs (Table 1). In general, the guidelines aim at preventing potential effects on soil productivity and fertility, due to disturbance of physicochemical properties of soil, and possible risks for human health, through the presence of toxic compounds and pathogens (Aquarec, 2006; EPA, 2012; WHO, 2006a). However, the impact of wastewater irrigation on soil ecosystem services, which relies on an adequate equilibrium of diversity and activity of soil microbiota, crucial for soil health (Anderson, 2003; Torsvik and Ovreas, 2002), may be not properly covered by the available guidelines. Another relevant omission refers to the risks of introduction of biological contaminants, such as pathogens and antibiotic resistance genes, through the transmission pathways environment-plants-humans.

Much attention has been given to the potential of different wastewater treatment technologies to overcome the emerging challenges, as the removal of novel classes of contaminants or the sustainable reuse of wastewater for irrigation. In particular, pharmaceutical products, pesticides and disinfectants are foresighted in most of the discussions around wastewater treatment and quality (Michael et al., 2013; Pal et al., 2014; Rivera-Utrilla et al., 2013). A recent literature review by Norton-Brandão et al. (2013) offers a comprehensive overview of the technologies commonly used to treat wastewater reused for irrigation, making a comparison based on the parameter's salinity, pathogens, nutrients and heavy metals. The use of technologies based on sedimentation, filtration or disinfection processes, such as chlorine dioxide, UV, ozone or TiO_2 seems adequate for wastewater treatment for irrigation, depending on the

raw wastewater quality and application demands (Norton-Brandão et al., 2013). Regarding the removal of microorganisms, membrane bioreactor (MBR) systems show high efficiency, meeting also other relevant objectives such as the removal of heavy metals. Other processes such as constructed wetlands, ponds and disinfection oxidants may also offer good removal rates of microorganisms, although may be not so efficient on the achievement of adequate levels of other parameters, in particular salinity (Norton-Brandão et al., 2013). The choice of the best methods for the wastewater treatments must represent a compromise between cost effectivity and the production of water with adequate quality for irrigation. In this respect, nowadays, binding quality criteria should include also the absence of emerging contaminants as pharmaceuticals or antibiotic resistant bacteria. Since the implementation and maintenance costs and the environmental impacts cannot be ignored, some- times it may be challenging to achieve an ideal compromise.

3. Influence of wastewater irrigation on soil microbial communities

Soil microbial communities are established on basis of a complex network of interrelations between abiotic (physical and chemical soil properties) and biotic factors (macro- and microbiological soil components). The impact of wastewater on soil microbial communities is supposed to depend on direct inputs of exogenous microbiota, which, in an improbable worst case scenario, would lead to the elimination of autochthonous microorganisms by competition. In addition, and probably not less relevant, are the indirect effects produced by wastewater, which may contribute to change the physicochemical soil properties and, therefore, induce microbial community disturbances. Both types of impact are almost uncharacterized, constituting deep gaps of knowledge. To the best of our knowledge, up to now few studies have applied commonly used methodological approaches to assess microbial community structure and activity on the evaluation of effects of wastewater irrigation on the soil microbiota (Table 2). However, nowadays, some methodological limitations are becoming easier to overcome thanks to the multiple applications of the next generation sequencing methods. In the next sections the direct and indirect effects that wastewater irrigation may have on soil microbiota and, therefore, on its properties will be discussed.

3.1. Physicochemical soil properties versus soil microbiota

Several physicochemical parameters, such as pH or organic matter content, have been shown to influence both abiotic and biotic soil parameters (Table 3). Because wastewater irrigation has the potential to cause variation in those physicochemical parameters, possible effects on soil properties and microbiota will be discussed in the next sections.

3.1.1. pH

Soil pH influences the availability of nutrients and metals, the cation exchange capacity, as well as the mineralisation of organic matter (Table 3). The increase of soil pH was observed after long-term irrigation with wastewater (4 to 60 years), in soils with distinct management regimens (arable or in grazed pastoral soils) and irrigated with different types of wastewater (secondary-treated municipal wastewater and dairy wastewater) (Table 4). In opposition, the decrease of soil pH was reported in agricultural soils dedicated to lettuce, livestock fodder and orange production irrigated with wastewater for more than 15, 20 and 40 years, respectively (Table 4). Although the effects on soil microbiota due to the abovementioned pH variations were not explored in those studies, this parameter seems to be an important determinant of the richness (number of different species) and diversity (variety of organisms) of soil bacterial communities (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). In a comprehensive study comparing soil bacterial communities from distinct ecosystems it was observed that communities of sites with identical pH values share similar indices of bacterial diversity and richness, irrespective of other factors such as climate conditions or edaphic properties (Fierer and Jackson, 2006). In general, soil habitats with pH values in the neutrality range tend to present a higher bacterial diversity than those more acidic or alkaline (Fierer and Jackson, 2006; Lauber et al., 2009). In contrast, fungal communities may be not so vulnerable to pH variations (Rousk et al., 2010). Variations on pH can also influence the solubility of different soil components, such as metals (Bloom, 2000; Sparks, 2003) (Table 3). The increase of free metals in soil irrigated with wastewater was related to a decrease of soil pH (Rattan et al., 2005). In turn, the concentration and availability of metals have the potential to affect the microbial communities (Bååth, 1989; Brookes, 1995; Chander and Brookes, 1991; Müller et al., 2002).

3.1.2. Organic matter

Soil organic matter is essential as nutrient reservoir and in soil structure. Through the formation and stabilisation of aggregates, organic matter content contributes for the capacity of soil to retain water, affecting the drainage properties and resistance to compaction. Organic matter is also a reservoir of nutrients (such as N, P and S) important for plant growth, providing also a substantial part of the soil's cation exchange capacity (CEC) (Baldock and Nelson, 2000; Powlson et al., 2013; Sparks, 2003) (Table 3). In this way, organic matter content is of vital importance for soil fertility.

The organic matter-related pools are amongst the soil properties most affected by wastewater irrigation, as has been suggested by different studies reporting an increase of total organic C and/or N in irrigated soil (Table 4). Depending on the type of organic matter inputs, an increase in the organic matter availability may promote the selection of specific bacterial populations (Sun et al., 2014; Torsvik and Ovreas, 2002). For instance, the supplying of easily metabolisable nutrients may contribute to select bacterial groups with high growth rates, such as members of the classes *Alpha-Proteobacteria* and *Beta-Proteobacteria* (Smit et al., 2001). Effects on soil aggregate stability and water retention capacity due to inputs of organic matter from irrigation with wastewater has been described (Levy et al., 2013; Vogeler, 2008). However, these effects may depend on the concentration and composition of organic matter and on

soil texture. While in loamy sand soils wastewater irrigation led to an increase in the aggregate stability, in sandy clay and clay soils resulted in a decrease (Levy et al., 2013). The characteristics of the soil aggregates are very important, since 40-70% of the soil bacteria are associated with stable microaggregates and clay particles with sizes lower than 20 μm (Ranjard and Richaume, 2001). Therefore, the interference on the formation of soil aggregates due to wastewater irrigation is expected to modify soil microhabitats and, hence, influence the soil microbial communities (Torsvik and Ovreas, 2002). In general, the increase of soil organic matter was associated with a positive effect on soil structure and water retention (Baldock and Nelson, 2000; Sparks, 2003). However, this is not a general rule, and the opposite effect may be observed. The coating of soil particles with organic matter or even microbial biofilms can, in some circumstances, promote the hydrophobicity of the soil and therefore the water repellence (Doerr et al., 2000; Nadav et al., 2013; Tarchitzky et al., 2007). The complexity of implications of wastewater irrigation limits the establishment of possible cause-effect relationships, and the supplying of organic matter is of this a good example. Although most of the studies report an increase on organic matter due to wastewater irrigation, some did not detect significant variations but still reported alterations on microbiological and biochemical parameters (Table 4). The influence of organic matter supply on the microbiota is suggested by the fact that wastewater irrigation was sometimes associated with an increase in soil microbial biomass and in soil enzyme activities (Table 4). Examples are the activities of enzymes such as dehydrogenase, laccase, cellulase, protease and urease (Chevremont et al., 2013; Friedel et al., 2000; Liu and Haynes, 2011; Morugán-Coronado et al., 2013). These effects are probably due to the stimulation of microbial growth and activity, boosted by the organic matter and nutrients supplied by wastewater (Adrover et al., 2012; Chevremont et al., 2013; Friedel et al., 2000; Liu and Haynes, 2011; Morugán-Coronado et al., 2013; Tam, 1998).

3.1.3. Nitrogen, phosphorus and other plant nutrients

The availability of plant nutrients such as N, P or K is essential for plant growth (Haygarth et al., 2013; White and Greenwood, 2013). One of the advantages of wastewater irrigation is the supplying of nutrients that may be sufficient to replace, or at least to reduce, the use of synthetic fertilizers in agriculture (Adrover et al., 2012; Fatta-Kassinos et al., 2011; Toze, 2006). The fertilizing potential of the wastewater (assuming an application rate of 5000 m^3/ha year) can reach annual values as high as 250 kg/ha for N, 50 kg/ha for P and 150 kg/ha for K (Pescod, 1992). In addition, wastewater can also be a source of other macro- and micronutrients such as Ca, Mg, B, Mg, Fe, Mn or Zn (Henze and Comeau, 2008). Indeed, irrigation with wastewater has been shown to significantly increase the yields of non-edible crops such as lemon grass or rosebushes (Lal et al., 2013; Marinho et al., 2013), and to have a positive impact on oil's quality recovered from sunflower and castor crops (Tsoutous et al., 2013).

The increase of different N forms, i.e. $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ or total N, has been observed

after wastewater irrigation of orchards (citrus or hazel), sugarcane, corn, forage crops and mangrove for less than 1 year up to 20 years (Table 4). Identically, long term wastewater irrigation contributes to increase the available P (Table 4). Both N and P contents may impact soil microbial communities, in particular the activity associated with the cycling of these elements. Tam (1998) observed a concomitant increase of inorganic forms of N ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) and of ammonia and nitrite-oxidizing bacterial counts in wastewater irrigated soils. Similar findings were reported by Habteselassie et al. (2013) when N sources, from different origins including dairy wastes, were added to agricultural soils. N availability is also reported as able to interfere, either increasing or decreasing the bacterial richness. Gelsomino et al. (2006) reported that the increase of total N observed in soils flooded with wastewater was concomitant with a decrease in the genetic diversity of ammonia-oxidizing bacteria when compared to controls. However, the addition of ammonium nitrate to soils was shown to increase the bacterial richness (Turlapati et al., 2013). Friedel et al. (2000) studying a soil irrigated with wastewater for more than 18 years demonstrated that denitrifying activity was stimulated comparing to those under rainfed agriculture. This difference was probably due to the availability of $\text{NO}_3\text{-N}$ released as product of the activity ammonia and nitrite-oxidizing bacterial groups.

Despite the possible benefits for crop productivity, the excessive provision of nutrients in soil may have adverse effects. Nutrients such as available P and nitrate may be leached into the surface and groundwater, causing eutrophication or toxicity in other habitats (Candela et al., 2007; Knobeloch et al., 2000; Wu, 1999) (Table 3). The excess of nutrients can also disturb the autochthonous soil microbial communities. For example, the accumulation of inorganic-N in soils may affect the microbial catabolic activity, in particular the biodegradation of recalcitrant carbon compounds present in soil (DeForest et al., 2004; Ramírez et al., 2012).

3.1.4. Salinity

Wastewater contains higher concentrations of dissolved inorganic substances, including soluble salts, than freshwater. Therefore, wastewater irrigation may promote soil salinisation (increase of soluble salts concentration) or sodification (increase of sodium ions relative to other cations). In turn, salinisation and sodification are also associated with the increase of electrical conductivity. These are the most commonly reported negative effects caused by wastewater irrigation (EPA, 2012; Pescod, 1992; WHO, 2006a) (Table 4). Excessive soil salinity imposes hyperosmotic, oxidative stress and ion toxicity, constituting a limiting factor for plant growth, development and productivity (Levy and Tai, 2013; Ngara et al., 2012). On the other hand, sodification affects negatively the stability of soil aggregates and soil structure, leading to an increase of soil compaction, loss of soil permeability and reduction of hydraulic conductivity (Sparks, 2003) (Table 3). These factors will interfere not only with plant growth but also with soil microbiota. Effects on microbial communities are mainly related with the alteration of the soil structure and with the decrease of osmotic potential (Chowdhury et al., 2011; Sarig et al., 1993; Torsvik and Ovreas, 2002; Wong et al., 2008). Increase in soil salinity has been shown to reduce fungal and bacterial counts (Omar et al., 1994; Pankhurst et al., 2001), as well as, to reduce microbial diversity and microbial biomass (Ke et al., 2013; Tripathi et al., 2006). Rietz and Haynes

(2003) related salinity and sodicity with increased levels of microbial stress and a reduction on the metabolical efficiency of the microbial community. Indeed, soil salinity seems to influence the C and N mineralization, and the retardation of nitrification (Azam and Ifzal, 2006; Sarig et al., 1993).

3.1.5. Contaminants

Wastewater transports different types of contaminants (e.g., metals, organic micropollutants), which through irrigation may accumulate in soil. These contaminants may diffuse or propagate to the surrounding environment and in soil may hinder its fertility and/or disturb the microbial communities (Table 3). The incapacity of the soil ecosystem to eliminate contaminants supplied by biosolids or water has been designated terraccumulation (Rooklidge, 2004) (Table 3). Metals such as Fe, Cr, Zn, Pb, Ni, Cd and Cu, abundant in wastewater, are on the top list of potential contaminants accumulating due to wastewater irrigation (Table 4). Besides the potential phytotoxicity and consequent effects on plant growth and/or contamination, metals may also disturb the autochthonous microbial communities (e.g. reduction of microbial biomass or alteration of the community structure, Table 3). In addition, microbial functions impaired by metal contamination, include C and N mineralization, soil enzyme activity and litter decomposition (Bååth, 1989; Brookes, 1995; Chander and Brookes, 1991; Müller et al., 2002) (Table 3). Some studies reporting metal accumulation in soil after irrigation with wastewater related this accumulation with the reduction of alkaline phosphatase activity and soil ATP content, the suppression of the sporulation and diversity alteration of arbuscular mycorrhizal fungi and the emergence of metal resistance in bacteria (Faryal et al., 2007; Ortega-Larrocea et al., 2001; Yim and Tam, 1999). Leaching is another possible consequence of metal accumulation in soils, mainly after long periods (~20 years) of irrigation with wastewater (Xu et al., 2010). Moreover, accumulated metals may interact synergistically with other contaminants, such as antibiotics, exacerbating their potential effects (Kong et al., 2006; Peltier et al., 2010). Boron is a semi-metallic element and also a pollutant commonly present in wastewater (EPA, 2012; WHO, 2006a). Besides its toxicity to human and plants (Nable et al., 1997; Parks and Edwards, 2005), B may also affect microbial communities, although the reported effects due to its excess in soils are scarce in literature and commonly associated with effects of soil salinity. Nevertheless, B has been shown to reduce bacterial diversity and dehydrogenase activity of soils (Ibekwe et al., 2010; Khan et al., 2012; Nelson and Mele, 2007).

Organic contaminants transported by wastewater, such as phenolic compounds, surfactants, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceutical products may also accumulate in soils due to long-term irrigation with wastewater. These compounds may increase soil toxicity and represent a potential ecological risk in the soil ecosystem (Fatta-Kassinos et al., 2011; Song et al., 2006b). Potential adverse effects include also the disturbance of soil microbiota (Table 3). Phenolic compounds may promote changes in the soil microbial community structure and function, for instance through the stimulation of primary phenol degraders and trophically related populations (DeRito et al., 2005; Sinsabaugh, 2010). Surfactants, such as linear alkylbenzene sulfonate (LAS), are other common wastewater contaminants, which accumulation in soil may have adverse effects. Although these effects are not always observed and depend on the surfactant type (Abu-Zreig et al., 2003; Sklarz et al., 2013), their accumulation may generate water-repellent soils, with effects in hydraulic properties and crop

productivity (Doerr et al., 2000; Wiel-Shafran et al., 2006). In addition, changes in the structure of soil bacterial communities due to surfactant contamination have been reported (Sánchez-Peinado et al., 2010). Also the exposure to PCBs is capable of diminishing soil bacterial abundance (Correa et al., 2010), and phenanthrene, a model compound for PAHs, has negative effects on several bacterial groups, reducing soil richness and evenness (Ding et al., 2012).

Endocrine-disruptors and a myriad of pharmaceutical products (e.g. antimicrobials, personal care products, lipid regulator agents, anti-inflammatory drugs, betablockers, cancer therapeutics, contraceptives and other hormones) are contaminants of emerging concern (Chen et al., 2011; Fatta-Kassinos et al., 2011; Kinney et al., 2006; Muñoz et al., 2009; Shi et al., 2012; Thiele-Bruhn, 2003; Toze, 2006). The fate and effect of these compounds in soils will depend on several key factors, which go far behind the chemical properties of each one or of the soil characteristics. Compounds with low mobility, such as the antimicrobials ciprofloxacin, sulfamethoxazole, and carbamazepine, were shown to accumulate in soils with the irrigation period (0–100 years), in contrast with others highly mobile (diclofenac, naproxen, bezafibrate) (Chefetz et al., 2008; Dalkmann et al., 2012; Gibson et al., 2010; Kinney et al., 2006).

Antimicrobial compounds in soils may disturb the structure and activity of microbial communities (Ding and He, 2010; Kong et al., 2006; Liu et al., 2012; Müller et al., 2002) (Table 3). Indeed, disturbances on N transformation, methanogenesis, and sulphate reduction were reported (Ding and He, 2010). Oxytetracycline and sulfamethoxazole have been shown to decrease the functional diversity of soil microbial community (Kong et al., 2006; Liu et al., 2012), and tylosin, to alter the bacterial structure of soil (Müller et al., 2002). Such effects may be more pronounced for antimicrobials with high tendency to adsorb to soil particles, since adsorption promotes the concentration of the compound without loss of activity (Chander et al., 2005). Some antimicrobial compounds are known to suffer degradation in soils (Lin and Gan, 2011; Monteiro and Boxall, 2009; Xu et al., 2009; Zhang et al., 2012). However, highly mobile contaminants and/or degradation products thereof are not of less concern, since due to leaching processes can contaminate groundwater. For instance, amoxicillin degradation products were observed to contaminate groundwater in an agricultural field irrigated with wastewater (Gozlan et al., 2013).

The discussion about the effects of environmental contaminants is often oversimplified since most of the times a single compound and target function or parameter are assessed. However, contaminants seldom occur alone in wastewater and, thus, the combination of different compounds may make the prediction of possible effects, even more complex. It is arguable that in soil, antimicrobial compounds may interact with other contaminants. Indeed, it is documented that antibiotics may favour the soil adsorption of metals such as Cu and Cd, reducing their toxicity to plants (Jia et al., 2008; Wan et al., 2010). In turn, metals can also enhance the effects of antibiotics accumulation in soils (Kong et al., 2006; Peltier et al., 2010). In comparison with the individual contaminants, the combination of oxytetracycline and Cu was observed to significantly decrease the bacterial diversity, evenness and soil microbial community function (Kong et al., 2006). Last but not least, the possibility that these pollutants contribute for antibiotic resistance selection cannot be ignored (Chee-Sanford et al., 2009; Wang et al., 2014).

3.2. Effects on microbial abundance and activity

The increase of soil microbial biomass due to wastewater irrigation was observed in several studies (Table 4). As described above, this effect may be due to the provision of organic carbon, as is suggested by the simultaneous increase in the activity of dehydrogenase, a parameter generally indicative of biological oxidation of organic compounds (Alguacil et al., 2012; Elifantz et al., 2011; Frenk et al., 2014; Friedel et al., 2000) (Table 2). Through the supplying of organic matter and nutrients, soil irrigation with wastewater is expected to stimulate different organisms and metabolic pathways. The increase in the activity of some enzymes (e.g. hydrolytic, proteolytic, laccases, cellulases, phosphatase) has been reported in soils irrigated with treated wastewater (Adrover et al., 2012; Alguacil et al., 2012; Chevremont et al., 2013; Elifantz et al., 2011; Frenk et al., 2014; Friedel et al., 2000; Meli et al., 2002; Morugán-Coronado et al., 2013). It is, thus, suggested that wastewater irrigation may stimulate the activity of microorganisms involved in the biochemical balance of elements such as C, N and P. In addition, organic matter may stabilize the enzymes, which remain active in the extra-cellular medium, independently of the soil microbial (Trasar-Cepeda et al., 2008a). However, these modifications are not necessarily beneficial and the stimulation of the soil microbial abundance and activity may have negative impacts on soil properties. For instance, Magesan et al. (1999) observed that the bacterial growth stimulated by irrigation with wastewater led to the formation of biofilms, with the concomitant clogging of the pore spaces between particles, with implications in the soil hydraulic conductivity. Again, the complexity of the cause-effect relationships associated with wastewater irrigation is emphasized. In the literature available it is not possible to find a general agreement about the promotion of microbial abundance and activity in soils irrigated with wastewater. Indeed, either a decrease or no noticeable effects on the microbial biomass or enzymatic activity were reported by several authors (Table 4). Tam (1998) did not observe changes in phosphatase or dehydrogenase activities in mangrove soils irrigated with wastewater. Kayikcioglu (2012) reported a decrease of the activities of enzymes aryl sulfatase, dehydrogenase, urease, alkaline phosphatase and β -glucosidase in wastewater irrigated agricultural soil. The inhibition of microbial growth or activity after wastewater irrigation is probably explained by the fact that besides nutrients also contaminants, such as heavy metals, are being supplied (Kayikcioglu, 2012; Yim and Tam, 1999).

4. Autochthonous versus exogenous microbiota

4.1. Soil and wastewater bacterial diversity

Soil and wastewater have quite different characteristics, but both are inhabited by a wide diversity of bacteria. A first glance to evaluate the likelihood of disturbances in soil microbial communities due to the introduction of exogenous bacteria through irrigation with wastewater can be given by a comparative diversity assessment (Fig. 1). Most of the recent studies on the bacterial diversity of these habitats are based on culture independent methods (16S rRNA gene-PCR-DGGE, clone libraries and high-throughput sequencing methods). Although these approaches may offer a reliable overview of the bacterial community, microbial identification is only possible above the genus level, a fact that hinders a thorough comparison of the microbiota of soil and wastewater.

A summary of studies reporting the bacterial diversity of agricultural soils and treated wastewaters is shown in Fig. 1 and Table S1. Due to the lack of a consensual soil classification, the data herein reported for agricultural soils is independent of soil texture, edaphic characteristics and type of crops. The data of treated wastewater include those treated in lagoons and/or in wastewater treatment plants, treating municipal, industrial, including pharmaceutical, and/or animal wastewater.

At the phylum level, *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Acidobacteria* and *Verrucomicrobia* are amongst the most abundant bacterial groups thriving in both types of habitat (Fig. 1, Table S1). In contrast, some bacterial groups commonly found in treated wastewater are seldom, if ever, detected in agricultural soil. This was the case for the phyla *Deinococcus-Thermus*, *Thermotogae*, *Synergistetes* or for members of the orders *Methylophilales*, *Neisseriales*, *Alteromonadales*, *Methylococcales*, *Thiotrichales*, *Vibrionales*, *Desulfobacterales*, *Erysipelotrichales*, *Selenomonadales*, *Holophagales*, *Opitutales*, *Puniceococcales*, *Phycisphaerales*, *Planctomycetales*, and *Spirochaetales* (Fig. 1, Table S1). Not surprisingly, some of these bacteria comprise members of the human microbiome, i.e. are commensal or pathogenic bacteria, and were detected in the human gastrointestinal or urogenital tracts (*Vibrionales*, *Erysipelotrichales*, *Selenomonadales*, *Spirochaetales*) (NIH Human Microbiome Project catalog, <http://www.hmpdacc.org/catalog/>). It is thus suggested that, if not outcompeted, bacteria of human origin may become part of the soil microbiome through the use of treated wastewater for irrigation. Although some bacteria inhabiting wastewater are ubiquitous and may occur in different habitats, their passage through the human body may increase the chances of acquisition of exogenous genetic material. In this respect, mobile genetic elements, antibiotic resistance and virulence genes are of special concern. For instance, members of the orders *Aeromonadales*, *Alteromonadales*, *Enterobacteriales*, *Legionellales*, *Pseudomonadales*, *Vibrionales*, *Xanthomonadales*, *Actinomycetales*, *Bacillales*, *Clostridiales*, *Lactobacillales* and *Bacteroidales* are common members of the human microbiome (NIH Human Microbiome Project catalog, <http://www.hmpdacc.org/catalog/>) and described as carriers of multiple determinants of antibiotic resistance (Vaz-Moreira et al., 2014).

On the other hand, the use of wastewater for irrigation may be a source of beneficial bacteria for the soils. For example, the introduction of bacteria involved in the N cycling or other with the ability to remediate soil contaminants (e.g. pesticides, heavy metals or antibiotics) may contribute for the improvement of the soil quality (Hanjra et al., 2012; Oved et al., 2001). Oved et al. (2001) reported that soils irrigated with treated wastewater were enriched with different *Nitrosomonas* like species. Although bacterial groups such as the ammonia and nitrite oxidizing bacteria (e.g. *Nitrosomonas* and *Nitrobacter* or *Nitrospirae*, respectively) are not frequently detected in bacterial diversity studies of treated wastewaters (Table S1, Fig. 1), they may eventually be enriched in soils explaining the results of Oved et al. (2001).

One of the challenges concerning the microbiological impacts of wastewater irrigation is to understand if and how the introduced micro-organisms will affect the soil microbial community. These effects may be related not only with the interference that exogenous populations may have on the soil microbial community, but also with the capacity of the exogenous organisms to survive in soil and constitute a health risk to humans and livestock. To the best of our knowledge,

there are no studies reporting the fate of wastewater microorganisms introduced in soil through irrigation, neither describing their influence on the soil native microbial communities. Because plant and human health risks posed by wastewater irrigation are relevant issues, some inferences based on the literature available are the subject of the next subsections.

4.2. Risk of dissemination of pathogens through wastewater reuse

The occurrence of pathogens in treated domestic wastewater is well documented (Okoh et al., 2007; Varela and Manaia, 2013) and their transmission to humans by direct contact or through the food chain is of concern (Hussain et al., 2002; Solomon et al., 2002; Steele and Odumeru, 2004; Wachtel et al., 2002). In addition, indirect transmission pathways include the air, due to the formation of aerosols, or water, due to runoff or leaching (Hussain et al., 2002). The risks posed to humans by pathogens transmitted through wastewater irrigation are difficult to estimate, but will depend, amongst other factors, on the survival of pathogens in the environment, the infective dose, and the host immunity (Hussain et al., 2002; Shuval and Fattal, 2003). In the environment, in particular in soil, the survival of exogenous microorganisms, including pathogens, depends on factors such as the antagonism with native microbiota, moisture content, organic matter, pH, and temperature (Brandl, 2006; Shuval and Fattal, 2003). The survival periods in soil or crops for some pathogenic bacteria and parasites may vary from only few days (e.g. *Campylobacter* spp.) up to one year for the highly resistant eggs of the helminth *Ascaris* (Shuval and Fattal, 2003), or much longer periods for fungi spores (e.g. *Hemileia vastatrix*, *Puccinia melanocephala*, *Puccinia striiformis* f. sp. *tritici*) or highly resistant bacterial endospores (e.g. *Bacillus cereus*, *Clostridium botulinum* or *Clostridium perfringens*) produced by some phyto- and human pathogens, respectively (Brown and Hovmoller, 2002; Brown, 2000; Harris et al., 2003).

When considering transmission of human pathogens through the food chain, one important parameter to be considered is the type of plants produced by the irrigated soil and the method employed for irrigation. For fruit trees or vegetables cultivated on vines and not in direct contact with irrigation water, the risks of transmission may be lower than for vegetables which grow in direct contact with the soil and irrigation wastewater (Cirelli et al., 2012; Melloul et al., 2001). In addition, methods that increase the probability of direct contact between wastewater and edible part of plants (e.g., furrow irrigation) seem to promote greater contamination of plants than subsurface drip irrigation (Song et al., 2006a). However, Christou et al. (2014) reused a tertiary treated effluent for irrigation of tomato crops and did not find evidences of microbiological contamination of the edible parts.

Rhizosphere is a habitat for plant-beneficial bacteria, however it may also host potential human pathogens (Berg et al., 2005; Holden et al., 2013). Bacterial genera such as *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Ochrobactrum*, *Pseudomonas*, *Ralstonia*, *Staphylococcus* and *Stenotrophomonas* are inhabitants of plant roots also able to colonize humans (Berg et al., 2005). Besides colonizing the vegetables surface, some bacteria may live inside the plants (endophytes). A positive correlation between plant contamination and irrigation with wastewater has been described, suggesting that wastewater can be an important source of bacteria that will colonize plants (Armon et al., 1994; Howard and Hutcheson, 2003; Ibenyassine et al., 2006; Tyler and Triplett,

2008). Part of these bacteria can be transmitted to the consumers, mainly through uncooked vegetables. Opportunistic human pathogens described as endophytic bacteria include members of the genus *Staphylococcus* and family *Enterobacteriaceae* (e.g., *Hafnia*, *Yersinia*, *Pantoea*, *Salmonella*, *Enterobacter*, *Citrobacter* and *Klebsiella*) (Markova et al., 2005; Opelt et al., 2007; Rosenblueth and Martínez-Romero, 2006; Sturz et al., 2000; Wang et al., 2006). Although some *Enterobacteriaceae* have the ability to promote plant growth (Rosenblueth and Martínez-Romero, 2006; Tyler and Triplett, 2008), risks for human health cannot be ignored. Species comprising opportunistic pathogens such as *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* were detected in vegetables after irrigation with wastewater (Al-Lahham et al., 2003; Ibenyassine et al., 2007). A good example is the enterohemorrhagic strain *E. coli* O157:H7 that can be internalized into lettuce when exposed to contaminated irrigation water or soil (Solomon et al., 2002; Wachtel et al., 2002). Moreover, because plants are privileged hosts for these bacteria, if supplied in irrigation water, they can proliferate and survive for longer periods when plants are present than in their absence (Gagliardi and Karns, 2002; Ibekwe et al., 2004; Tyler and Triplett, 2008). Another risk is that bacteria transmitted by this pathway may be inherited through plant seeds, as has been described for some endophytic bacteria (Burnett et al., 2000; Cooley et al., 2003; Guo et al., 2001; Rosenblueth and Martínez-Romero, 2006; Tyler and Triplett, 2008; Wang et al., 2006). The risks associated with the dissemination of human pathogens due to the use of treated wastewater for irrigation can be considered low (Bichai et al., 2012; Shuval and Fattal, 2003). However, a pathway of transmission, comprising treated wastewater, soil-vegetables and human consumers, can be defined and should be considered when the implications of wastewater irrigation are discussed.

Another possible adverse effect of wastewater irrigation is the introduction of phytopathogens in the soils. Although the risks of propagation of phytopathogens to soils through wastewater are scarcely addressed in literature, it is recognised that several plant pathogens, either bacteria, fungi, viruses, parasitic nematodes or oomycetes (e.g. *Phytophthora* and *Pythium*) are waterborne (Bush et al., 2003; Hong and Moorman, 2005). Evidences of possible risks are supported by reports involving the use of recycled freshwater in plant nurseries and greenhouses, concluding that this can be identified as an important source and vehicle for the spread of plant pathogens (Stewart-Wade, 2011). Also the cross checking of potential phytopathogens in the list of microorganisms normally occurring in treated wastewater, suggests some candidates. Bacterial phytopathogens found in irrigation systems include *Corynebacterium flaccumfaciens*, *Erwinia* spp., *Pseudomonas syringae*, *Ralstonia solanacearum* and *Xanthomonas* spp. (Hong and Moorman, 2005). Some members of these genera (e.g. *Pseudomonas* and *Xanthomonas*) and other plant pathogens, such as members of the genera *Acidovorax* and *Herbaspirillum* and plant viruses (families *Tombusviridae*, *Geminiviridae*, *Nanoviridae* and the genus *Tobamovirus*) (Alhamlan et al., 2013; Fegan, 2006; Rosario et al., 2009) are also common inhabitants of treated wastewater. The scant information on the presence of phytopathogens in treated wastewater hampers decisive conclusions on the risks posed by wastewater irrigation. However, the introduction of plant pathogens through irrigation with wastewater cannot be ruled out until further studies are conducted focusing this issue.

4.3. Risks and precautions associated with antibiotic resistance

Treated wastewater contains high loads of bacteria. Most of these are of environmental origin and a non-negligible fraction (up to 10^3 colony forming units per mL) is derived from human and animal guts (Novo and Manaia, 2010; Varela and Manaia, 2013). Inevitably, these bacteria harbour antibiotic resistance genes, frequently associated with mobile genetic elements, which means that these genes have a high potential to be propagated amongst the bacterial community (Rizzo et al., 2013; Vaz-Moreira et al., 2014). In part this situation can be attributed to the fact that although conventional secondary wastewater treatment processes can reduce the cultivable bacterial loads around 2 logarithmic cycles, it fails to reduce the prevalence of antibiotic resistance and, sometimes, can even contribute for its increase (Ferreira da Silva et al., 2006; Luczkiewicz et al., 2010; Manaia et al., 2010; Novo et al., 2013; Rizzo et al., 2013; Zhang et al., 2009). This means that treated wastewater will contain high doses of antibiotic resistant bacteria. An estimate made for ciprofloxacin resistant coliforms, suggests that, per minute, a treatment plant will discharge 10^9 resistant bacteria (Vaz-Moreira et al., 2014). Over the years, antibiotic resistance has accumulated in the environment, humans and other animals. In particular old antibiotics such as aminopenicillins, sulfonamides, tetracyclines or erythromycin are nowadays inactive against bacterial groups formerly susceptible to those drugs. The prevalence of resistance to these old antibiotics can reach more than 50% of some bacterial populations discharged in the final effluents of wastewater treatment plants with conventional treatment (Manaia et al., 2012; Rizzo et al., 2013). The time elapsed between the emergence of a new resistance gene in clinical settings and its detection in municipal wastewater is frighteningly short (Szczepanowski et al., 2009). For instance, bacteria resistant to last resort antibiotics mainly or exclusively used in hospitals, such as carbapenems resistant Gram-negative bacteria, vancomycin resistant enterococci (VRE), or methicillin resistant *Staphylococcus aureus* (MRSA) are nowadays detected in municipal wastewater worldwide (Manaia et al., 2012; Rizzo et al., 2013; Baker-Austin et al., 2006; Fluit and Schmitz, 2004; Hernández et al., 1998; Miyahara et al., 2011). The wide contamination of the environment and the food chain with these genetic determinants may represent a public health calamity. Even if no emerging resistance types occur in wastewater, the dose of bacteria resistant to currently used and old antibiotics that will be discharged in an irrigated field is very high, and the possibility that these organisms can proliferate in soils and/or plants cannot be over ruled. The fate of antibiotic resistant bacteria and resistance genes in soils after wastewater irrigation is still poorly understood. Even though some wastewater bacteria will be outcompeted in soil, two types of negative consequences can be anticipated: i) some antibiotic resistant populations can proliferate in soil or plants, behaving as invasive species; and ii) some antibiotic resistance genes can be horizontally transferred (by conjugation, transduction or transformation) from wastewater bacteria to soil or plant bacteria. Hypothetically, both can take place simultaneously. However, the studies available do not strongly support those effects. Instead, the few publications available on the subject, show some controversial findings. While Negreanu et al. (2012) and Gatica and Cytryn (2013) failed to find any evidences that antibiotic resistant bacteria from treated wastewater could compete or survive in soils after for 6–8 years of irrigation, Wang et al. (2014) reported opposite results (Table 4). Wang et al. (2014) observed higher diversity and abundance of antibiotic resistance genes in soils irrigated with wastewater

than in a pristine soil. Furthermore, soils irrigated with wastewater presented higher abundance of the gene encoding a class 1 integrase (*int11*). Confirming the hypothesis that the abundance of the gene *int11* could indicate a high potential for horizontal gene transfer in soils, Wang et al. (2014) observed significant positive correlations between that gene and genes encoding resistance for tetracycline and sulfonamide (*tetG*, *sul1*, and *sul2*). One of the concerns associated with the continuous discharges of antibiotic resistant bacteria in soils is the high stability that some resistance genotypes may have. Although it might be generally assumed that antibiotic resistant bacteria or their genes can only persist if they have a competitive advantage, being selected by the presence of antibiotics, it has been demonstrated that this is not the case. Indeed, even in the absence of selective pressures or at concentrations found in many natural environments antibiotic resistant bacteria can be maintained or even enriched (Andersson and Hughes, 2010; Gullberg et al., 2011; Heuer et al., 2008). In addition, antibiotic resistance genes may occur in the environment also as naked DNA. It is known that extracellular DNA easily adsorbs to the sediment matrix and organic matter, prevalent in soils, and when adsorbed, DNA may be more resistant against DNase I degradation than the free naked DNA (Crecchio and Stotzky, 1998; Lorenz and Wackernagel, 1994). Hence, the persistence of antibiotic resistance genes in the soils irrigated with wastewater will depend mainly on the ability of the host bacteria to survive and proliferate in the receiving habitat or on the ability of the native microbiota to acquire the naked DNA fragments and proliferate after the acquisition. Although it is still very difficult to predict what may be the effect of the naked DNA in the soil, it was possible to demonstrate that antibiotic resistance genes (from both intra- and extracellular DNA) could be detected in soil in high doses by quantitative PCR after 16 months of environmental exposure (Hong et al., 2013; Selvam et al., 2012). These arguments show that even if immediate impacts are not observed, long term effects of accumulation of resistant bacteria, resistance genes and antibiotic residues should not be ignored (Dalkmann et al., 2012).

The apparent controversial results on the detection of contaminant antibiotic resistance in soil due to wastewater irrigation can be attributed to several factors, which obviously include the quality of the waste- water and of the soil. However, it is important to note that the detection of soil contamination with antibiotic resistance genes and/or bacteria in soil is technically challenging. In first place, since antibiotic resistance is a natural property of bacteria that can be observed in natural communities, it is critical to define in each case the resistance back- ground naturally existing in soil in order to assess the level of contamination coming from wastewater (Cytryn, 2013; Knapp et al., 2010). On the other hand, contamination by antibiotic resistant bacteria or genes may occur at very low levels, which in spite of the potential biological impact (e.g. facilitating horizontal gene transfer) may be below the quantification limits of the techniques commonly used (e.g. qPCR of DNA extracts produced from less than one gram of soil).

Antibiotic resistance is increasingly recognised as a global threat for human health and efforts to contain the dissemination of resistance genes and resistant bacteria are urgently needed (WHO, 2014). Wastewater reuse for irrigation poses a serious risk of contamination of soils, ground- and surface water, the wild life and the food-chain. Therefore, the current state of knowledge alerts for the need to apply the precautionary principle, i.e. avoid the irrigation with wastewater containing high levels of antibiotic resistance, as those normally resultant from most conventional wastewater treatment processes. In this respect, it seems advisable to assume that advanced treatment processes may be required for a safe wastewater reuse in irrigation.

5. Conclusions

The use of wastewater for irrigation is regarded as a way to address the imbalance between water demand and water supply. However the literature shows that irrigation with treated wastewater is not exempt of implications, some of them adverse. Alterations, such as the increase of organic matter related pools, salinity and soil accumulation of contaminants are the most commonly reported effects, with several examples being found in literature. Also the responses of soil microbiota to wastewater irrigation are varied and include the increase of microbial biomass and activity and different types of alterations on the microbial community structure. However, the most evident outcome of the literature search is that the effects on soil microbiota are neglected in the majority of studies on irrigation with wastewater. This is a major gap in the knowledge, given the importance of soil microbiota on soil health and fertility. A critical review of the current knowledge on soil and wastewater microbiology gives unequivocal indications that wastewater irrigation, in spite of the unquestionable benefits, may have adverse impacts on both physicochemical and microbiological properties of the soil. These will influence soil fertility and productivity, raising important concerns about the sustainability of continued reuse of treated waste- water in agriculture. Studies on the effects on soil of wastewater irrigation are challenging, since direct cause-effect relationships can hardly be found. It is suggested that multi-parametric analyses and holistic studies may bring additional and reliable insights into this problem.

The risks posed for human health represent another question at the heart of any discussion on wastewater reuse. These risks cannot be accurately estimated at the moment, but cannot be ignored. The evidences reported in the literature, as well as the critical analyses on the limitations of some experimental approaches highlight the importance of the accumulation and propagation of biological contaminants in soils due to wastewater irrigation. Human and animal pathogens, phytopathogens and antibiotic resistant bacteria and their genes are important biological contaminants that can be transported by wastewater and/or be enriched in soil. Also numerous chemical contaminants, included in categories such as xenobiotics, pharmaceuticals and metals, can threaten the environmental and human health. The mixture of these contaminants may have unpredictable consequences in both environmental and human health.

For a sustainable and safe wastewater reuse, parameters affecting both soil health and safety should be included in the quality standards, avoiding the disturbance of soil properties and the dissemination of emerging chemical and biological contaminants. Simultaneously, affordable technological solutions with minimal environmental impacts must be developed in order to assure wastewater treatment processes compatible with sustainable uses.

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References

Abu-Zreig, M., Rudra, R.P., Dickinson, W.T., 2003. Effect of application of surfactants on hydraulic properties of soils. *Biosyst. Eng.* 84, 363-372.

Adrover, M., Farrus, E., Moya, G., Vadell, J., 2012. Chemical properties and biological activity in soils of Mallorca following twenty years of treated wastewater irrigation. *J. Environ. Manag.* 95, S188-S192.

Aleem, A., Isar, J., Malik, A., 2003. Impact of long-term application of industrial wastewater on the emergence of resistance traits in *Azotobacter chroococcum* isolated from rhizospheric soil. *Bioresour. Technol.* 86, 7-13.

Alguacil, M.M., Torrecillas, E., Torres, P., García-Orenes, F., Roldán, A., 2012. Long-term effects of irrigation with waste water on soil AM fungi diversity and microbial activities: the implications for agro-ecosystem resilience. *PLoS One* 7, e47680.

Alhamlan, F.S., Ederer, M.M., Brown, C.J., Coats, E.R., Crawford, R.L., 2013. Metagenomics-based analysis of viral communities in dairy lagoon wastewater. *J. Microbiol. Methods* 92, 183-188.

Al-Lahham, O., El Assi, N.M., Fayyad, M., 2003. Impact of treated wastewater irrigation on quality attributes and contamination of tomato fruit. *Agric. Water Manag.* 61, 51-62.

Anderson, T.-H., 2003. Microbial eco-physiological indicators to assess soil quality. *Agric. Ecosyst. Environ.* 98, 285-293.

Andersson, D.I., Hughes, D., 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat. Rev. Microbiol.* 8, 260-271.

Aquarec Project, 2006. Work Package 2: Guideline for Quality Standards for Water Reuse in Europe. EVK1-CT-2002-00130.

Arlosoroff, S., 2007. Wastewater management, treatment, and reuse in Israel. In: Zaidi, M.K. (Ed.), *Wastewater Reuse – Risk Assessment, Decision-making and Environmental Security*. Springer, Netherlands, pp. 55-64.

Armon, R., Dosoretz, C.G., Azov, Y., Shelef, G., 1994. Residual contamination of crops irrigated with effluent of different qualities – a field-study. *Water Sci. Technol.* 30, 239-248.

Ayers, R.S., Westcot, D.W., 1994. *Water quality for agriculture – FAO irrigation and drainage paper 29 Rev.1*. Food and Agriculture Organization of the United Nation – FAO, Rome, Italy (ISBN 92-5-102263-10).

Azam, F., Ifzal, M., 2006. Microbial populations immobilizing $\text{NH}^+\text{-N}$ and NO^- -N differ in their sensitivity to sodium chloride salinity in soil. *Soil Biol. Biochem.* 38, 2491-2494.

Bååth, E., 1989. Effects of heavy-metals in soil on microbial processes and populations (a review). *Water Air Soil Pollut.* 47, 335-379.

- Bai, Q., Gattinger, A., Zelles, L., 2000. Characterization of microbial consortia in paddy rice soil by phospholipid analysis. *Microb. Ecol.* 39, 273-281.
- Baker-Austin, C., Wright, M.S., Stepanauskas, R., McArthur, J.V., 2006. Co-selection of anti-biotic and metal resistance. *Trends Microbiol.* 14, 176-182.
- Baldock, J.A., Nelson, P.N., 2000. Soil organic matter. In: Sumner, M.E. (Ed.), *Handbook of Soil Science*. CRC Press LLC, USA, pp. B25-B84.
- Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., et al., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J.* 6, 248-258.
- Berg, G., Eberl, L., Hartmann, A., 2005. The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ. Microbiol.* 7, 1673-1685.
- Bichai, F., Polo-López, M.I., Fernández Ibañez, P., 2012. Solar disinfection of wastewater to reduce contamination of lettuce crops by *Escherichia coli* in reclaimed water irrigation. *Water Res.* 46, 6040-6050.
- Bixio, D., Thoeye, C., De Koning, J., Joksimovic, D., Savic, D., Wintgens, T., et al., 2006. Wastewater reuse in Europe. *Desalination* 187, 89-101.
- Bloom, P.R., 2000. Soil pH and pH buffering. In: Sumner, M.E. (Ed.), *Handbook of Soil Science*. CRC Press LLC, USA, pp. B333-B352.
- Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microb. Ecol.* 36, 1-12.
- Brandl, M.T., 2006. Fitness of human enteric pathogens on plants and implication for food safety. *Annu. Rev. Phytopathol.* 44, 367-392.
- Brookes, P.C., 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. *Biol. Fertil. Soils* 19, 269-279.
- Brown, K.L., 2000. Control of bacterial spores. *Br. Med. Bull.* 56, 158-171.
- Brown, J.K., Hovmoller, M.S., 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* 297, 537-541.
- Brzezinska, M., Tiwari, S.C., Stepniewska, Z., Nosalewicz, M., Bennicelli, R.P., Samborska, A., 2006. Variation of enzyme activities, CO₂ evolution and redox potential in an Eutric Histosol irrigated with wastewater and tap water. *Biol. Fertil. Soils* 43, 131-135.
- Burnett, S.L., Chen, J.R., Beuchat, L.R., 2000. Attachment of *Escherichia coli* O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. *Appl. Environ. Microbiol.* 66, 4679-4687.
- Bush, E., Hong, C., Stromberg, E., 2003. Fluctuations of *Phytophthora* and *Pythium* spp. in components of a recycling irrigation system. *Plant Dis.* 87, 1500-1506.
- Candela, L., Fabregat, S., Josa, A., Suriol, J., Vigués, N., Mas, J., 2007. Assessment of soil and groundwater impacts by treated urban wastewater reuse. A case study: application in a golf course (Girona, Spain). *Sci. Total Environ.* 374, 26-35.
- Chander, K., Brookes, P.C., 1991. Effects of heavy-metals from past applications of sewage-sludge on microbial biomass and organic-matter accumulation in a sandy loam and silty loam UK soil. *Soil Biol. Biochem.* 23, 927-932.

Chander, Y., Kumar, K., Goyal, S.M., Gupta, S.C., 2005. Antibacterial activity of soil-bound antibiotics. *J. Environ. Qual.* 34, 1952-1957.

Chee-Sanford, J.C., Mackie, R.I., Koike, S., Krapac, I.G., Lin, Y.F., Yannarell, A.C., et al., 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J. Environ. Qual.* 38, 1086-1108.

Chefetz, B., Mualem, T., Ben-Ari, J., 2008. Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater. *Chemosphere* 73, 1335-1343.

Chen, F., Ying, G.G., Kong, L.X., Wang, L., Zhao, J.L., Zhou, L.J., et al., 2011. Distribution and accumulation of endocrine-disrupting chemicals and pharmaceuticals in wastewater irrigated soils in Hebei, China. *Environ. Pollut.* 159, 1490-1498.

Cheng, F., Peng, X., Zhao, P., Yuan, J., Zhong, C., Cheng, Y., et al., 2013. Soil microbial biomass, basal respiration and enzyme activity of main forest types in the Qinling mountains. *PLoS One* 8, e67353.

Chevremont, A.C., Boudenne, J.L., Coulomb, B., Farnet, A.M., 2013. Impact of watering with UV-LED-treated wastewater on microbial and physico-chemical parameters of soil. *Water Res.* 47, 1971-1982.

Chowdhury, N., Marschner, P., Burns, R.G., 2011. Soil microbial activity and community composition: impacts of changes in matric and osmotic potential. *Soil Biol. Biochem.* 43, 1229-1236.

Christou, A., Maratheftis, G., Eliadou, E., Michael, C., Hapeshi, E., Fatta-Kassinos, D., 2014. Impact assessment of the reuse of two discrete treated wastewaters for the irrigation of tomato crop on the soil geochemical properties, fruit safety and crop productivity. *Agric. Ecosyst. Environ.* 192, 105-114.

Cirelli, G.L., Consoli, S., Licciardello, F., Aiello, R., Giuffrida, F., Leonardi, C., 2012. Treated municipal wastewater reuse in vegetable production. *Agric. Water Manag.* 104, 163-170.

Cooley, M.B., Miller, W.G., Mandrell, R.E., 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. *Appl. Environ. Microbiol.* 69, 4915-4926.

Correa, P.A., Lin, L., Just, C.L., Hu, D., Hornbuckle, K.C., Schnoor, J.L., et al., 2010. The effects of individual PCB congeners on the soil bacterial community structure and the abundance of biphenyl dioxygenase genes. *Environ. Int.* 36, 901-906.

Crecchio, C., Stotzky, G., 1998. Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. *Soil Biol. Biochem.* 30, 463-470.

Cytryn, E., 2013. The soil resistome: the anthropogenic, the native, and the unknown. *Soil Biol. Biochem.* 63, 18-23.

Dalkmann, P., Broszat, M., Siebe, C., Willaschek, E., Sakinc, T., Huebner, J., et al., 2012. Accumulation of pharmaceuticals, *Enterococcus*, and resistance genes in soils irrigated with wastewater for zero to 100 years in central Mexico. *PLoS One* 7,

e45397.

DeAngelis, K.M., Allgaier, M., Chavarria, Y., Fortney, J.L., Hugenholtz, P., Simmons, B., et al., 2011. Characterization of trapped lignin-degrading microbes in tropical forest soil. *PLoS One* 6, e19306.

Decreto Ministeriale, 15/2003, 2003. Regolamento recante norme tecniche per il riutilizzo delle acque reflue in attuazione dell'articolo 26, comma 2, del decreto legislativo 11 maggio 1999. Ministero dell'Ambiente e della Tutela del Territorio, Italia.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in Northern Hardwood forests. *Soil Sci. Soc. Am. J.* 68, 132-138.

DeRito, C.M., Pumphrey, G.M., Madsen, E.L., 2005. Use of field-based stable isotope probing to identify adapted populations and track carbon flow through a phenol-degrading soil microbial community. *Appl. Environ. Microbiol.* 71, 7858-7865.

Ding, C., He, J., 2010. Effect of antibiotics in the environment on microbial populations. *Appl. Microbiol. Biotechnol.* 87, 925-941.

Ding, G.-C., Heuer, H., Smalla, K., 2012. Dynamics of bacterial communities in two unpolluted soils after spiking with phenanthrene: soil type specific and common responders. *Front. Microbiol.* 3, 290. <http://dx.doi.org/10.3389/fmicb.2012.00290>.

Doerr, S.H., Shakesby, R.A., Walsh, R.P.D., 2000. Soil water repellency: its causes, characteristics and hydro-geomorphological significance. *Earth-Sci. Rev.* 51, 33-65.

Eickhorst, T., Tippkötter, R., 2008. Improved detection of soil microorganisms using fluorescence in situ hybridization (FISH) and catalyzed reporter deposition (CARD-FISH). *Soil Biol. Biochem.* 40, 1883-1891.

Eichorst, S.A., Kuske, C.R., Schmidt, T.M., 2011. Influence of plant polymers on the distribution and cultivation of bacteria in the phylum Acidobacteria. *Appl. Environ. Microbiol.* 77, 586-596.

Elifantz, H., Kautsky, L., Mor-Yosef, M., Tarchitzky, J., Bar-Tal, A., Chen, Y., et al., 2011. Microbial activity and organic matter dynamics during 4 years of irrigation with treated wastewater. *Microb. Ecol.* 62, 973-981.

EMWIS, 2007. Mediterranean Wastewater Reuse Report. Mediterranean Wastewater Reuse Working Group (MED WWR WG), (<http://www.emwis.net/topics/>).

EPA, 2012. Guidelines for Water Reuse. Environmental Protection Agency (EPA), Washington DC (EPA/600/R-12/618).

EU, 2007. Addressing the challenge of water scarcity and droughts in the European Union.

European Union (EU), Brussels (COM/2007/0414).

FAO, 2008. Drylands, people and land use. In: Koohafkan, P., Stewart, B.A. (Eds.), *Water and Cereals in Drylands*. Food and Agriculture Organization of the

United Nations (FAO), Roma. ISBN: 978-92-5-1060520, pp. 5-16.

FAO, 2012. Coping with water scarcity. An action framework for agriculture and food security. FAO Water Reports. Food and Agriculture Organization of the United Nations (FAO), Rome. ISBN: 978-92-5-107304-9.

FAO, 2013. FAO statistical yearbook 2013. World Food and Agriculture. Food and Agriculture Organization of the United Nations (FAO), Rome 978-92-5-107396-4.

Faryal, R., Tahir, F., Hameed, A., 2007. Effect of wastewater irrigation on soil along with its micro and macro flora. Pak. J. Bot. 39, 193-204.

Fatta-Kassinos, D., Kalavrouziotis, I.K., Koukoulakis, P.H., Vasquez, M.I., 2011. The risks associated with wastewater reuse and xenobiotics in the agroecological environment. Sci. Total Environ. 409, 3555-3563.

Fegan, M., 2006. Plant pathogenic members of the genera *Acidovorax* and *Herbaspirillum*. In: Gnanamanickam, S.S. (Ed.), Plant-associated Bacteria. Springer, Dordrecht, The Netherlands, pp. 671-702.

Ferreira da Silva, M., Tiago, I., Verissimo, A., Boaventura, R.A., Nunes, O.C., Manaia, C.M., 2006. Antibiotic resistance of enterococci and related bacteria in an urban wastewater treatment plant. FEMS Microbiol. Ecol. 55, 322-329.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. 103, 626-631.

Fluit, A.C., Schmitz, F.J., 2004. Resistance integrons and super-integrons. Clin. Microbiol. Infect. 10, 272-288.

Frenk, S., Hadar, Y., Minz, D., 2014. Resilience of soil bacterial community to irrigation with water of different qualities under Mediterranean climate. Environ. Microbiol. 16, 559-569.

Friedel, J.K., Langer, T., Siebe, C., Stahr, K., 2000. Effects of long-term waste water irrigation on soil organic matter, soil microbial biomass and its activities in central Mexico. Biol. Fertil. Soils 31, 414-421.

Gagliardi, J.V., Karns, J.S., 2002. Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. Environ. Microbiol. 4, 89-96.

Gatica, J., Cytryn, E., 2013. Impact of treated wastewater irrigation on antibiotic resistance in the soil microbiome. Environ. Sci. Pollut. Res. 20, 3529-3538.

Gelsomino, A., Badalucco, L., Ambrosoli, R., Crecchio, C., Puglisi, E., Meli, S.M., 2006. Changes in chemical and biological soil properties as induced by anthropogenic disturbance: a case study of an agricultural soil under recurrent flooding by wastewaters. Soil Biol. Biochem. 38, 2069-2080.

Gibson, R., Durán-Álvarez, J.C., Estrada, K.L., Chávez, A., Jiménez Cisneros, B., 2010. Accumulation and leaching potential of some pharmaceuticals and potential endocrine disruptors in soils irrigated with wastewater in the Tula Valley, Mexico. Chemosphere 81, 1437-1445.

Gozlan, I., Rotstein, A., Avisar, D., 2013. Amoxicillin-degradation products formed under controlled environmental conditions: identification and determination in the aquatic environment. Chemosphere 91, 985-992.

- Gullberg, E., Cao, S., Berg, O.G., Ilback, C., Sandegren, L., Hughes, D., et al., 2011. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.* 7, e1002158.
- Guo, X., Chen, J.R., Brackett, R.E., Beuchat, L.R., 2001. Survival of *Salmonellae* on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Appl. Environ. Microbiol.* 67, 4760-4764.
- Habteselassie, M.Y., Xu, L., Norton, J.M., 2013. Ammonia-oxidizer communities in an agricultural soil treated with contrasting nitrogen sources. *Front. Microbiol.* 4 (e2013. 00326).
- Hanjra, M.A., Blackwell, J., Carr, G., Zhang, F., Jackson, T.M., 2012. Wastewater irrigation and environmental health: implications for water governance and public policy. *Int. J. Hyg. Environ. Health* 69, 215-255.
- Hansel, C.M., Fendorf, S., Jardine, P.M., Francis, C.A., 2008. Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. *Appl. Environ. Microbiol.* 74, 1620-1633.
- Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., et al., 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf.* 2, 78-141.
- Hayat, S., Ahmad, I., Azam, Z.M., Ahmad, A., Inam, A., Samiullah, 2002. Effect of long-term application of oil refinery wastewater on soil health with special reference to microbiological characteristics. *Bioresour. Technol.* 84, 159-163.
- Haygarth, P.M., Bardgett, R.D., Condon, L.M., 2013. Nitrogen and phosphorus cycles and their management. In: Gregory, P.J., Nortcliff, S. (Eds.), *Soil Conditions and Plant Growth*. Wiley-Blackwell, West Sussex, UK, pp. 132-159.
- Haynes, R.J., Naidu, R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. *Nutr. Cycl. Agroecosyst.* 51, 123-137.
- He, Z., Gentry, T.J., Schadt, C.W., Wu, L., Liebich, J., Chong, S.C., et al., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *ISME J.* 1, 67-77.
- Helbling, D.E., Ackermann, M., Fenner, K., Kohler, H.-P.E., Johnson, D.R., 2012. The activity level of a microbial community function can be predicted from its metatranscriptome. *ISME J.* 6, 902-904.
- Henry, S., Bru, D., Stres, B., Hallet, S., Philippot, L., 2006. Quantitative detection of the *nosZ* gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, *narG*, *nirK*, and *nosZ* genes in soils. *Appl. Environ. Microbiol.* 72, 5181-5189.
- Henze, M., Comeau, Y., 2008. Wastewater characterization. In: Henze, M., van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D. (Eds.), *Biological Wastewater Treatment: Principles Modelling and Design*. IWA Publishing, London, UK, pp.

33-52.

Hernández, A., Mellado, R.P., Martínez, J.L., 1998. Metal accumulation and vanadium-induced multidrug resistance by environmental isolates of *Escherichia hermannii* and *Enterobacter cloacae*. *Appl. Environ. Microbiol.* 64, 4317-4320.

Heuer, H., Abdo, Z., Smalla, K., 2008. Patchy distribution of flexible genetic elements in bacterial populations mediates robustness to environmental uncertainty. *FEMS Microbiol. Ecol.* 65, 361-371.

Hidri, Y., Bouziri, L., Maron, P.-A., Anane, M., Jedidi, N., Hassan, A., et al., 2010. Soil DNA evidence for altered microbial diversity after long-term application of municipal wastewater. *Agron. Sustain. Dev.* 30, 423-431.

Hjort, K., Lembke, A., Speksnijder, A., Smalla, K., Jansson, J.K., 2007. Community structure of actively growing bacterial populations in plant pathogen suppressive soil. *Microb. Ecol.* 53, 399-413.

Holden, N.J., Pritchard, L., Wright, K., Toth, I.K., 2013. Mechanisms of plant colonization by human pathogenic bacteria: an emphasis on the roots and rhizosphere. In: de Bruijn,

F.J. (Ed.), *Molecular Microbial Ecology of the Rhizosphere* vol. 2. John Wiley and Sons, Hoboken, New Jersey, pp. 1217-1226.

Hong, C.X., Moorman, G.W., 2005. Plant pathogens in irrigation water: challenges and opportunities. *Crit. Rev. Plant Sci.* 24, 189-208.

Hong, P.Y., Yannarell, A.C., Dai, Q.H., Ekizoglu, M., Mackie, R.I., 2013. Monitoring the perturbation of soil and groundwater microbial communities due to pig production activities. *Appl. Environ. Microbiol.* 79, 2620-2629.

Hopkins, D.W., Sparrow, A.D., Shillam, L.L., English, L.C., Dennis, P.G., Novis, P., et al., 2008. Enzymatic activities and microbial communities in an Antarctic dry valley soil: responses to C and N supplementation. *Soil Biol. Biochem.* 40, 2130-2136.

Hori, T., Muller, A., Igarashi, Y., Conrad, R., Friedrich, M.W., 2010. Identification of iron-reducing microorganisms in anoxic rice paddy soil by ^{13}C -acetate probing. *ISME J.* 4, 267-278.

Howard, M.B., Hutcheson, S.W., 2003. Growth dynamics of *Salmonella enterica* strains on alfalfa sprouts and in waste seed irrigation water. *Appl. Environ. Microbiol.* 69, 548-553. Hussain, I., Raschid, L., Hanjra, M.A., Marikar, F., van der Hoek, W., 2002. Wastewater use in agriculture: review of impacts and methodological issues in valuing impacts.

Working Paper 37. International Water Management Institute, Colombo, Sri Lanka.

Ibekwe, A.M., Watt, P.M., Shouse, P.J., Grieve, C.M., 2004. Fate of *Escherichia coli* O157:H7 in irrigation water on soils and plants as validated by culture method and real-time PCR. *Can. J. Microbiol.* 50, 1007-1014.

Ibekwe, A.M., Poss, J.A., Grattan, S.R., Grieve, C.M., Suárez, D., 2010. Bacterial diversity in cucumber (*Cucumis sativus*) rhizosphere in response to salinity, soil pH and boron. *Soil Biol. Biochem.* 42, 567-575.

Ibenyassine, K., AitMhand, R., Karamoko, Y., Cohen, N., Ennaji, M.M., 2006. Use of repetitive DNA sequences to determine the persistence of enteropathogenic *Escherichia coli* in vegetables and in soil grown in fields treated with contaminated irrigation water. *Lett. Appl. Microbiol.* 43, 528-533.

Ibenyassine, K., Mhand, R.A., Karamoko, Y., Anajjar, B., Chouibani, M., Ennaji, M.M., 2007. Bacterial pathogens recovered from vegetables irrigated by wastewater in Morocco. *J. Environ. Health* 69, 47-51.

Jia, D.-A., Zhou, D.-M., Wang, Y.-J., Zhu, H.-W., Chen, J.-L., 2008. Adsorption and cosorption of Cu(II) and tetracycline on two soils with different characteristics. *Geoderma* 146, 224-230. Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J.* 3, 442-453.

Kalavrouziotis, I.K., Kokkinos, P., Oron, G., Fatone, F., Bolzonella, D., Vatyliotou, M., et al., 2013. Current status in wastewater treatment, reuse and research in some Mediterranean countries. *Desalin. Water Treat.* 1-16.

Kayikcioglu, H.H., 2012. Short-term effects of irrigation with treated domestic wastewater on microbiological activity of a Vertic xerofluvent soil under Mediterranean conditions. *J. Environ. Manag.* 102, 108-114.

Ke, C., Li, Z., Liang, Y., Tao, W., Du, M., 2013. Impacts of chloride de-icing salt on bulk soils, fungi, and bacterial populations surrounding the plant rhizosphere. *Appl. Soil Ecol.* 72, 69-78.

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z., Zhu, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ. Pollut.* 152, 686-692.

Khan, R.U., Anderson, C.W.N., Loganathan, P., Xue, J., Clinton, P.W., 2012. Response of *Pinus radiata* D. Don to boron fertilization in a glasshouse study. *Commun. Soil Sci. Plant Anal.* 43, 1412-1426.

Kidd, P.S., Prieto-Fernández, A., Monterroso, C., Acea, M.J., 2008. Rhizosphere microbial community and hexachlorocyclohexane degradative potential in contrasting plant species. *Plant Soil* 302, 233-247.

Kinney, C.A., Furlong, E.T., Werner, S.L., Cahill, J.D., 2006. Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. *Environ. Toxicol. Chem.* 25, 317-326.

Knapp, C.W., Dolfing, J., Ehlert, P.A., Graham, D.W., 2010. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. *Environ. Sci. Technol.* 44, 580-587.

Knobeloch, L., Salna, B., Hogan, A., Postle, J., Anderson, H., 2000. Blue babies and nitrate-contaminated well water. *Environ. Health Perspect.* 108, 675-678.

Kong, W.D., Zhu, Y.G., Fu, B.J., Marschner, P., He, J.Z., 2006. The veterinary antibiotic oxy-tetracycline and Cu influence functional diversity of the soil microbial community. *Environ. Pollut.* 143, 129-137.

Kuramae, E., Gamper, H., van Veen, J., Kowalchuk, G., 2011. Soil and plant factors driving the community of soil-borne microorganisms across

chronosequences of secondary succession of chalk grasslands with a neutral pH. *FEMS Microbiol. Ecol.* 77, 285-294.

Lal, K., Yadav, K.D., Kaur, R., Bundela, Khan, M.I., Chaudhary, M., et al., 2013. Productivity, essential oil yield, and heavy metal accumulation in lemon grass (*Cymbopogon flexuosus*) under varied wastewater-groundwater irrigation regimes. *Ind. Crop. Prod.* 45, 270-278.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* 75, 5111-5120.

Leal, R.M.P., Firme, L.P., Herpin, U., da Fonseca, A.F., Montes, C.R., dos Santos Dias, C.T., et al., 2010. Carbon and nitrogen cycling in a tropical Brazilian soil cropped with sugarcane and irrigated with wastewater. *Agric. Water Manag.* 97, 271-276.

Letunic, I., Bork, P., 2007. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* 23, 127-128.

Letunic, I., Bork, P., 2011. Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. *Nucleic Acids Res.* 39, 1-4.

Levy, D., Tai, G.C.C., 2013. Differential response of potatoes (*Solanum tuberosum* L.) to salinity in an arid environment and field performance of the seed tubers grown with fresh water in the following season. *Agric. Water Manag.* 116, 122-127.

Levy, G.J., Lordian, A., Goldstein, D., Borisover, M., 2013. Soil structural indices' dependence on irrigation water quality and their association with chromophoric components in dissolved organic matter. *Eur. J. Soil Sci.* 65, 197-205.

Lin, K., Gan, J., 2011. Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. *Chemosphere* 83, 240-246.

Lin, X., Zhao, Y., Fu, Q., Umashankara, M.L., Feng, Z., 2008. Analysis of culturable and unculturable microbial community in bensulfuron-methyl contaminated paddy soils. *J. Environ. Sci.* 20, 1494-1500.

Liu, Y.Y., Haynes, R.J., 2011. Influence of land application of dairy factory effluent on soil nutrient status and the size, activity, composition and catabolic capability of the soil microbial community. *Appl. Soil Ecol.* 48, 133-141.

Liu, F., Wu, J., Ying, G.-G., Luo, Z., Feng, H., 2012. Changes in functional diversity of soil microbial community with addition of antibiotics sulfamethoxazole and chlortetracycline. *Appl. Microbiol. Biotechnol.* 95, 1615-1623.

Lopes, A.R., Faria, C., Prieto-Fernández, Á., Trasar-Cepeda, C., Manaia, C.M., Nunes, O.C., 2011. Comparative study of the microbial diversity of bulk paddy soil of two rice fields subjected to organic and conventional farming. *Soil Biol. Biochem.* 43, 115-125. Lopes, A.R., Manaia, C.M., Nunes, O.C., 2014. Bacterial community variations in an alfalfa-rice rotation system revealed by 16S rRNA gene 454-pyrosequencing. *FEMS Microbiol. Ecol.* 87, 650-663.

López-Gutiérrez, J.C., Toro, M., López-Hernández, D., 2004. Arbuscular mycorrhiza

and enzymatic activities in the rhizosphere of *Trachypogon plumosus* Ness. in three acid savanna soils. *Agric. Ecosyst. Environ.* 103, 405-411.

Lorenz, M.G., Wackernagel, W., 1994. Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Mol. Biol. Rev.* 58, 563-602.

Luczkiewicz, A., Jankowska, K., Fudala-Ksiazek, S., Olanczuk-Neyman, K., 2010. Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. *Water Res.* 44, 5089-5097.

Magesan, G.N., Williamson, J.C., Sparling, G.P., Schipper, L.A., Lloyd-Jones, A.R., 1999. Hydraulic conductivity in soils irrigated with wastewaters of differing strengths: field and laboratory studies. *Aust. J. Soil Res.* 37, 391-402.

Manaia, C.M., Novo, A., Coelho, B., Nunes, O.C., 2010. Ciprofloxacin resistance in domestic wastewater treatment plants. *Water Air Soil Pollut.* 208, 335-343.

Manaia, C.M., Vaz-Moreira, I., Nunes, O.C., 2012. Antibiotic resistance in waste water and surface water and human health implications. In: Barceló, D. (Ed.), *Emerging Organic Contaminants and Human Health*. Springer-Verlag, Berlin Heidelberg, pp. 173-212.

Marecos do Monte, M.H.F., 2007. Guidelines for good practice of water reuse for irrigation: Portuguese standard NP4434. In: Zaidi, M.K. (Ed.), *Wastewater Reuse – Risk Assessment, Decision-Making and Environmental Security*. Springer, Dordrecht, The Netherlands, pp. 253-265.

Marinho, L.E., Tonetti, A.L., Stefanutti, R., Coraucci Filho, B., 2013. Application of reclaimed wastewater in the irrigation of rosebushes. *Water Air Soil Pollut.* 224, 1669.

Marinho, L.E.d.O., Coraucci Filho, B., Roston, D.M., Stefanutti, R., Tonetti, A.L., 2014. Evaluation of the productivity of irrigated *Eucalyptus grandis* with reclaimed wastewater and effects on soil. *Water Air Soil Pollut.* 225, 1830-1838.

Markova, Y.A., Romanenko, A.S., Dukhanina, A.V., 2005. Isolation of bacteria of the family Enterobacteriaceae from plant tissues. *Microbiology* 74, 575-578.

Meli, S., Porto, M., Belligno, A., Bufo, S.A., Mazzatura, A., Scopa, A., 2002. Influence of irrigation with lagooned urban wastewater on chemical and microbiological soil parameters in a citrus orchard under Mediterranean condition. *Sci. Total Environ.* 285, 69-77.

Melloul, A.A., Hassani, L., Rafouk, L., 2001. Salmonella contamination of vegetables irrigated with untreated wastewater. *World J. Microbiol. Biotechnol.* 17, 207-209.

Metcalf, Eddy, I., 2003. *Wastewater Engineering. Treatment and Reuse*. McGraw-Hill. Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., et al., 2013.

Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. *Water Res.* 47, 957-995.

Miyahara, E., Nishie, M., Takumi, S., Miyanojara, H., Nishi, J., Yoshiie, K., et al., 2011. Environmental mutagens may be implicated in the emergence of drug-resistant microorganisms. *FEMS Microbiol. Lett.* 317, 109-116.

Monteiro, S.C., Boxall, A.B.A., 2009. Factors affecting the degradation of

pharmaceuticals in agricultural soils. *Environ. Toxicol. Chem.* 28, 2546-2554.

Morugán-Coronado, A., Arcenegui, V., García-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., 2013. Application of soil quality indices to assess the status of agricultural soils irrigated with treated wastewaters. *Solid Earth* 4, 119-127.

Müller, A.K., Westergaard, K., Christensen, S., Sorensen, S.J., 2002. The diversity and function of soil microbial communities exposed to different disturbances. *Microb. Ecol.* 44, 49-58.

Muñoz, I., Gómez-Ramos, M.J., Agüera, A., Fernández-Alba, A.R., García-Reyes, J.F., Molina-Díaz, A., 2009. Chemical evaluation of contaminants in wastewater effluents and the environmental risk of reusing effluents in agriculture. *Trends Anal. Chem.* 28, 676-694.

Nable, R.O., Bañuelos, G.S., Paull, J.G., 1997. Boron toxicity. *Plant Soil* 193, 181-198. Nadav, I., Tarchitzky, J., Chen, Y., 2013. Induction of soil water repellency following irrigation with treated wastewater: effects of irrigation water quality and soil texture. *Irrig. Sci.* 31, 385-394.

Ndour, N.Y.B., Baudoin, E., Guissé, A., Seck, M., Khouma, M., Brauman, A., 2008. Impact of irrigation water quality on soil nitrifying and total bacterial communities. *Biol. Fertil. Soils* 44, 797-803.

Negreanu, Y., Pasternak, Z., Jurkevitch, E., Cytryn, E., 2012. Impact of treated wastewater irrigation on antibiotic resistance in agricultural soils. *Environ. Sci. Technol.* 46, 4800-4808.

Nelson, D.R., Mele, P.M., 2007. Subtle changes in rhizosphere microbial community structure in response to increased boron and sodium chloride concentrations. *Soil Biol. Biochem.* 39, 340-351.

Ngara, R., Ndimba, R., Borch-Jensen, J., Jensen, O.N., Ndimba, B., 2012. Identification and profiling of salinity stress-responsive proteins in *Sorghum bicolor* seedlings. *J. Proteomics* 75, 4139-4150.

Niemczynowicz, J., 1999. Urban hydrology and water management – present and future challenges. *Urban Water* 1, 1-14.

NOM-001-ECOL-1996 que establece los límites máximos permisibles de contaminantes en las descargas de aguas residuales en aguas y bienes nacionales. Secretaría de Medio Ambiente y Recursos Naturales, Mexico.

NOR-SASP1013629A, 2010. Arrêté du 2 août 2010 relatif à l'utilisation d'eaux issues du traitement d'épuration des eaux résiduaires urbaines pour l'irrigation de cultures ou d'espaces verts. Ministère de la Santé et des Sports, France.

Norton-Brandão, D., Scherrenberg, S.M., van Lier, J.B., 2013. Reclamation of used urban waters for irrigation purposes – a review of treatment technologies. *J. Environ. Manag.* 122, 85-98.

Novo, A., Manaia, C.M., 2010. Factors influencing antibiotic resistance burden in municipal wastewater treatment plants. *Appl. Microbiol. Biotechnol.* 87, 1157-1166.

Novo, A., Andre, S., Viana, P., Nunes, O.C., Manaia, C.M., 2013. Antibiotic resistance, antimicrobial residues and bacterial community composition in urban

wastewater. *Water Res.* 47, 1875-1887.

NWQMS. National Water Quality Management Strategy, 2000. Guidelines for Sewage Systems. Use of Reclaimed Water. Agriculture and Resource Management Council of Australia and New Zealand.

Okoh, A.I., Odjadjare, E.E., Igbinsola, E.O., Osode, A.N., 2007. Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. *Afr. J. Biotechnol.* 6, 2932-2944.

Omar, S.A., Abdel-Sater, M.A., Khallil, A.M., Abd-Alla, M.H., 1994. Growth and enzyme activities of fungi and bacteria in soil salinized with sodium chloride. *Folia Microbiol.* 39, 23-28.

Opelt, K., Berg, C., Berg, G., 2007. The bryophyte genus *Sphagnum* is a reservoir for powerful and extraordinary antagonists and potentially facultative human pathogens. *FEMS Microbiol. Ecol.* 61, 38-53.

Ortega-Larrocea, M.P., Siebe, C., Bécard, G., Méndez, I., Webster, R., 2001. Impact of a century of wastewater irrigation on the abundance of arbuscular mycorrhizal spores in the soil of the Mezquital Valley of Mexico. *Appl. Soil Ecol.* 16, 149-157.

Oved, T., Shaviv, A., Goldrath, T., Mandelbaum, R.T., Minz, D., 2001. Influence of effluent irrigation on community composition and function of ammonia-oxidizing bacteria in soil. *Appl. Environ. Microbiol.* 67, 3426-3433.

Pal, A., He, Y., Jekel, M., Reinhard, M., Gin, K.Y., 2014. Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. *Environ. Int.* 71, 46-62.

Pankhurst, C.E., Yu, S., Hawke, B.G., Harch, B.D., 2001. Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. *Biol. Fertil. Soils* 33, 204-217.

Parks, J.L., Edwards, M., 2005. Boron in the environment. *Crit. Rev. Environ. Sci. Technol.* 35, 81-114.

Pedrero, F., Kalavrouziotis, I., Alarcón, J.J., Koukoulakis, P., Asano, T., 2010. Use of treated municipal wastewater in irrigated agriculture – review of some practices in Spain and Greece. *Agric. Water Manag.* 97, 1233-1241.

Peltier, E., Vincent, J., Finn, C., Graham, D.W., 2010. Zinc-induced antibiotic resistance in activated sludge bioreactors. *Water Res.* 44, 3829-3836.

Pescod, M.B., 1992. Wastewater treatment and use in agriculture – FAO irrigation and drainage paper 47. Food and Agriculture Organization of the United Nation (FAO), Rome, Italy 92-5-103135-5.

Powlson, D., Smith, P., De Nobili, M., 2013. Soil organic matter. In: Gregory, P.J., Nortcliff, S. (Eds.), *Soil Conditions and Plant Growth*. Blackwell, West Sussex, UK, pp. 86-131.

Radajewski, S., Webster, G., Reay, D.S., Morris, S.A., Ineson, P., Nedwell, D.B., et al., 2002. Identification of active methylo-troph populations in an acidic forest soil by stable-isotope probing. *Microbiology* 148, 2331-2342.

Ramírez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen

amendments on soil microbial communities and processes across biomes. *Glob. Chang. Biol.* 18, 1918-1927.

Ranjard, L., Richaume, A., 2001. Quantitative and qualitative microscale distribution of bacteria in soil. *Res. Microbiol.* 152, 707-716.

Rattan, R.K., Datta, S.P., Chhonkar, P.K., Suribabu, K., Singh, A.K., 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and ground-water—a case study. *Agric. Ecosyst. Environ.* 109, 310-322.

Real Decreto 1620/2007 de 7 de diciembre, por el que se establece el régimen jurídico de la reutilización de las aguas depuradas. Ministerio de la Presidencia, España.

Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. *Soil Biol. Biochem.* 35, 845-854.

Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M.A., Prados-Joya, G., Ocampo-Pérez, R., 2013. Pharmaceuticals as emerging contaminants and their removal from water. A review. *Chemosphere* 93, 1268-1287.

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M.C., et al., 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Sci. Total Environ.* 447, 345-360.

Roger, P.A., Ladha, J.K., 1992. Biological N₂ fixation in wetland rice fields – estimation and contribution to nitrogen-balance. *Plant Soil* 141, 41-55.

Rooklidge, S.J., 2004. Environmental antimicrobial contamination from terraccumulation and diffuse pollution pathways. *Sci. Total Environ.* 325, 1-13.

Rosario, K., Nilsson, C., Lim, Y.W., Ruan, Y., Breitbart, M., 2009. Metagenomic analysis of viruses in reclaimed water. *Environ. Microbiol.* 11, 2806-2820.

Rosenblueth, M., Martínez-Romero, E., 2006. Bacterial endophytes and their interactions with hosts. *Mol. Plant-Microbe Interact.* 19, 827-837.

Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., et al., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4, 1340-1351.

Rui, J., Peng, J., Lu, Y., 2009. Succession of bacterial populations during plant residue decomposition in rice field soil. *Appl. Environ. Microbiol.* 75, 4879-4886.

Rusan, M.J.M., Hinnawi, S., Rousan, L., 2007. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination* 215, 143-152.

Sánchez-Peinado, M.d., González-López, J., Martínez-Toledo, M.V., Pozo, C., Rodelas, B., 2010. Influence of linear alkylbenzene sulfonate (LAS) on the structure of *Alphaproteobacteria*, *Actinobacteria*, and *Acidobacteria* communities in a soil microcosm. *Environ. Sci. Pollut. Res.* 17, 779-790.

Sarig, S., Roberson, E.B., Firestone, M.K., 1993. Microbial activity soil-structure – response to saline water irrigation. *Soil Biol. Biochem.* 25, 693-697.

Selvam, A., Xu, D.L., Zhao, Z.Y., Wong, J.W.C., 2012. Fate of tetracycline, sulfonamide and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. *Bioresour. Technol.* 126, 383-390.

- Shi, Y., Gao, L., Li, W., Liu, J., Cai, Y., 2012. Investigation of fluoroquinolones, sulfonamides and macrolides in long-term wastewater irrigation soil in Tianjin, China. *Bull. Environ. Contam. Toxicol.* 89, 857-861.
- Shiklomanov, I.A., 1993. World fresh water resources. In: Gleick, P.H. (Ed.), *Water in Crisis: A Guide to the World's Fresh Water Resources*. Oxford University Press, New York, pp. 13-24.
- Shrestha, P.M., Kube, M., Reinhardt, R., Liesack, W., 2009. Transcriptional activity of paddy soil bacterial communities. *Environ. Microbiol.* 11, 960-970.
- Shuval, H., Fattal, B., 2003. Control of pathogenic microorganisms in wastewater recycling and reuse in agriculture. In: Mara, D., Horan, N. (Eds.), *The Handbook of Water and Wastewater Microbiology*. Elsevier, pp. 241-262.
- Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol. Biochem.* 42, 391-404.
- Sklarz, M.Y., Zhou, M., Ferrando Chavez, D.L., Yakirevich, A., Gillor, O., Gross, A., et al., 2013. Effect of treated domestic wastewater on soil physicochemical and microbiological properties. *J. Environ. Qual.* 42, 1226-1235.
- Smit, E., Leeftang, P., Gommans, S., van den Broek, J., van Mil, S., Wernars, K., 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl. Environ. Microbiol.* 67, 2284-2291.
- Solomon, E.B., Yaron, S., Matthews, K.R., 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.* 68, 397-400.
- Song, I., Stine, S.W., Choi, C.Y., Gerba, C.P., 2006a. Comparison of crop contamination by microorganisms during subsurface drip and furrow irrigation. *J. Environ. Eng.* 132, 1243-1248.
- Song, Y.F., Wilke, B.M., Song, X.Y., Gong, P., Zhou, Q.X., Yang, G.F., 2006b. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals (HMs) as well as their genotoxicity in soil after long-term wastewater irrigation. *Chemosphere* 65, 1859-1868.
- Sparks, D.L., 2003. *Environmental Soil Chemistry*, 2nd ed. Academic Press, California, USA.
- Steele, M., Odumeru, J., 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. *J. Food Prot.* 67, 2839-2849.
- Stewart-Wade, S.M., 2011. Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: their detection and management. *Irrig. Sci.* 29, 267-297.
- Sturz, A.V., Christie, B.R., Nowak, J., 2000. Bacterial endophytes: potential role in developing sustainable systems of crop production. *Crit. Rev. Plant Sci.* 19, 1-30.
- Sun, H., Terhonen, E., Koskinen, K., Paulin, L., Kasanen, R., Asiegbu, F.O., 2014. Bacterial diversity and community structure along different peat soils in boreal forest. *Appl. Soil Ecol.* 74, 37-45.
- Swartjes, F.A., 2011. *Dealing with Contaminated Sites. From Theory Towards*

Practical Application. Springer, The Netherlands.

Szczepanowski, R., Linke, B., Krahn, I., Gartemann, K.H., Gutzkow, T., Eichler, W., et al., 2009. Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. *Microbiology* 155, 2306-2319.

Tam, N.F.Y., 1998. Effects of wastewater discharge on microbial populations and enzyme activities in mangrove soils. *Environ. Pollut.* 102, 233-242.

Tarchitzky, J., Lerner, O., Shani, U., Arye, G., Lowengart-Aycicegi, A., Brener, A., et al., 2007. Water distribution pattern in treated wastewater irrigated soils: hydrophobicity effect. *Eur. J. Soil Sci.* 58, 573-588.

Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils—a review. *J. Plant Nutr. Soil Sci.* 166, 145-167.

Torsvik, V., Ovreas, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. *Curr. Opin. Microbiol.* 5, 240-245.

Toze, S., 2006. Reuse of effluent water—benefits and risks. *Agric. Water Manag.* 80, 147-159.

Trasar-Cepeda, C., Leirós, M.C., Seoane, S., Gil-Sotres, F., 2008a. Biochemical properties of soils under crop rotation. *Appl. Soil Ecol.* 39, 133-143.

Trasar-Cepeda, C., Leirós, M.C., Gil-Sotres, F., 2008b. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. *Soil Biol. Biochem.* 40, 2146-2155.

Tripathi, S., Kumari, S., Chakraborty, A., Gupta, A., Chakrabarti, K., Bandyopadhyay, B.K., 2006. Microbial biomass and its activities in salt-affected coastal soils. *Biol. Fertil. Soils* 42, 273-277.

Tsoutous, T., Chatzakis, M., Sarantopoulos, I., Nikologiannis, A., Pasadakis, N., 2013. Effect of wastewater irrigation on biodiesel quality and productivity from castor and sunflower oil seeds. *Renew. Energy* 57, 211-215.

Turlapati, S.A., Minocha, R., Bhiravarasa, P.S., Tisa, L.S., Thomas, W.K., Minocha, S.C., 2013. Chronic N-amended soils exhibit an altered bacterial community structure in Harvard Forest, MA, USA. *FEMS Microbiol. Ecol.* 83, 478-493.

Tyler, H.L., Triplett, E.W., 2008. Plants as a habitat for beneficial and/or human pathogenic bacteria. *Annu. Rev. Phytopathol.* 46, 53-73.

UNDP, 2006. Beyond Scarcity: Power, Poverty and the Global Water Crisis. United Nations Development Programme (UNDP), New York, USA 0-230-50058-7.

Van-Camp, L., Bujarrabal, B., Gentile, A.R., Jones, R.J.A., Montanarella, L., Olazabal, C., et al., 2004. Reports of the Technical Working Groups Established under the Thematic Strategy for Soil Protection. Office for Official Publications of the European Communities, Luxembourg (EUR 21319 EN/3).

Varela, A.R., Manaia, C.M., 2013. Human health implications of clinically relevant bacteria in wastewater habitats. *Environ. Sci. Pollut. Res.* 20, 3550-3569.

Vaz-Moreira, I., Nunes, O.C., Manaia, C.M., 2014. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. *FEMS Microbiol. Rev.* 38, 761-778. <http://dx.doi.org/10.1111/1574->

6976.12062.

Vogeler, I., 2008. Effect of long-term wastewater application on physical soil properties.

Water Air Soil Pollut. 196, 385-392.

Wachtel, M.R., Whitehand, L.C., Mandrell, R.E., 2002. Association of *Escherichia coli* O157: H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. J. Food Prot. 65, 18-25.

Wan, Y., Bao, Y., Zhou, Q., 2010. Simultaneous adsorption and desorption of cadmium and tetracycline on cinnamon soil. Chemosphere 80, 807-812.

Wang, Z., Chang, A.C., Wu, L., Crowley, D., 2003. Assessing the soil quality of long-term reclaimed wastewater-irrigated cropland. Geoderma 114, 261-278.

Wang, E.T., Tan, Z.Y., Guo, X.W., Rodríguez-Durán, R., Boll, G., Martínez-Romero, E., 2006. Diverse endophytic bacteria isolated from a leguminous tree *Conzattia multiflora* grown in Mexico. Arch. Microbiol. 186, 251-259.

Wang, F.H., Qiao, M., Lv, Z.E., Guo, G.X., Jia, Y., Su, Y.H., et al., 2014. Impact of reclaimed water irrigation on antibiotic resistance in public parks, Beijing, China. Environ. Pollut. 184, 247-253.

Wartiainen, I., Eriksson, T., Zheng, W., Rasmussen, U., 2008. Variation in the active diazotrophic community in rice paddy—nifH PCR-DGGE analysis of rhizosphere and bulk soil. Appl. Soil Ecol. 39, 65-75.

White, P.J., Greenwood, D.J., 2013. Properties and management of cationic elements for crop growth. In: Gregory, P.J., Nortcliff, S. (Eds.), Soil Conditions and Plant Growth. Wiley-Blackwell, pp. 160-194.

WHO, 2006a. Guidelines for the Safe Use of Wastewater, Excreta and Greywater vol. 2.

World Health Organization (WHO), France. ISBN: 92 4 154683 2.

WHO, 2006b. A Compendium for Standards for Wastewater Reuse in the Eastern Mediterranean Region. World Health Organisation (WHO), (WHO-EM/CEH/142/E).

WHO, 2014. Antimicrobial Resistance: Global Report on Surveillance. World Health Organization (WHO), Geneva 978-92-4-156474-8.

Widmer, F., Rasche, F., Hartmann, M., Fliessbach, A., 2006. Community structures and substrate utilization of bacteria in soils from organic and conventional farming systems of the DOK long-term field experiment. Appl. Soil Ecol. 33, 294-307.

Wiel-Shafran, A., Ronen, Z., Weisbrod, N., Adar, E., Gross, A., 2006. Potential changes in soil properties following irrigation with surfactant-rich greywater. Ecol. Eng. 26, 348-354.

Wong, V.N.L., Dalal, R.C., Greene, R.S.B., 2008. Salinity and sodicity effects on respiration and microbial biomass of soil. Biol. Fertil. Soils 44, 943-953.

Wu, R.S.S., 1999. Eutrophication, water borne pathogens and xenobiotic compounds: environmental risks and challenges. Mar. Pollut. Bull. 39, 11-22.

Xu, J., Wu, L., Chang, A.C., 2009. Degradation and adsorption of selected

pharmaceuticals and personal care products (PPCPs) in agricultural soils. *Chemosphere* 77, 1299-1305.

Xu, J., Wu, L., Chang, A.C., Zhang, Y., 2010. Impact of long-term reclaimed wastewater irrigation on agricultural soils: a preliminary assessment. *J. Hazard. Mater.* 183, 780-786.

Yao, H., Zhang, S., Xue, X., Yang, J., Hu, K., Yu, X., 2013. Influence of the sewage irrigation on the agricultural soil properties in Tongliao City, China. *Front. Environ. Sci. Eng.* 7, 273-280.

Yi, L., Jiao, W., Chen, X., Chen, W., 2011. An overview of reclaimed water reuse in China. *J. Environ. Sci.* 23, 1585-1593.

Yim, M.W., Tam, F.Y., 1999. Effects of wastewater-borne heavy metals on mangrove plants and soil microbial activities. *Mar. Pollut. Bull.* 39, 179-186.

Zhang, X.X., Zhang, T., Fang, H.H., 2009. Antibiotic resistance genes in water environment. *Appl. Microbiol. Biotechnol.* 82, 397-414.

Zhang, C.-L., Guo, X.-L., Li, B.-Y., Wang, Y., 2012. Biodegradation of ciprofloxacin in soil. *J. Mol. Liq.* 173, 184-186.

Zhang, C.-L., Guo, X.-L., Li, B.-Y., Wang, Y., 2012. Biodegradation of ciprofloxacin in soil. *J. Mol. Liq.* 173, 184-186.

Zhong, W.H., Cai, Z.C., 2007. Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay. *Appl. Soil Ecol.* 36, 84-91.

Zörb, C., Senbayram, M., Peiter, E., 2014. Potassium in agriculture – status and perspectives. *J. Plant Physiol.* 171, 656-669.

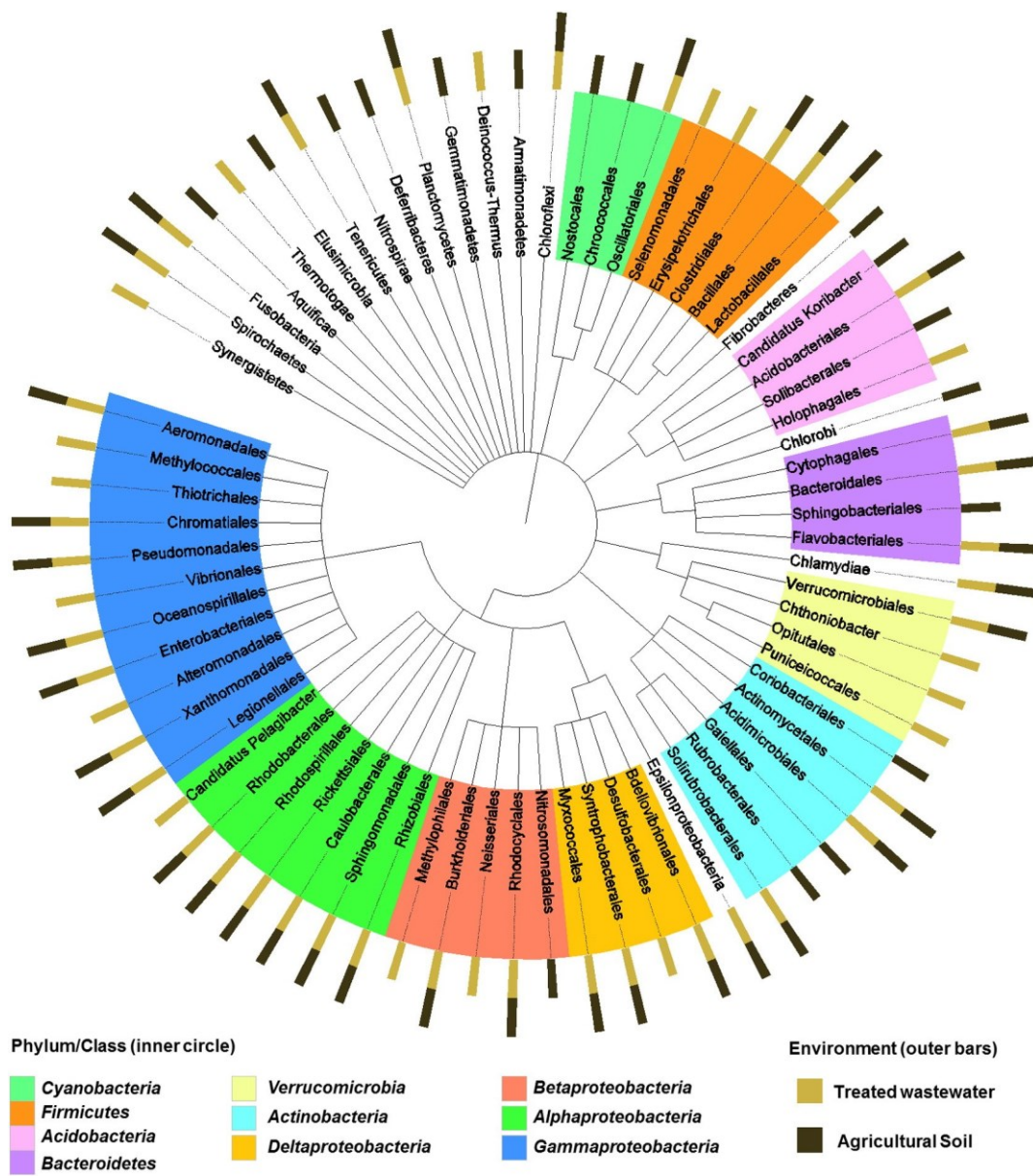


Fig. 1. Dendrogram representation of the bacterial diversity observed in treated wastewater and agricultural soil. The dendrogram was constructed with the iTOL – interactive tree of life (Letunic and Bork, 2007, 2011), based on the taxon ID codes, corresponding to the identifications provided in each of the publications cited (see Table S1).

Table 1
Guidelines on wastewater reuse for irrigation in different world regions.

Country or organism (year)	Irrigation categories	pH	EC (µS/cm)	SAR	NTU	SS (mg/L)	BOD (mg/L)	COD (mg/L)	DO (mg/L)	TNK or TN (mg/L)	N-NO ₃ (mg/L)	P (mg/L)	Sulphate (mg/L)	Total coliform (CFU/100 mL)	Faecal coliform (CFU/100 mL)	<i>Escherichia coli</i> (CFU/100 mL)	Nematode eggs (no./L)
US-EPA (2012) [1]	UR	6-9			2	30	10										
	R	6-9			-	-	10								Absent		
WHO (2006a) [2]	UR														2 × 10 ²	10 ³	≤1
	R															-	≤1
California (1978) [1]	ND	6-9			2				Present						2.2 × 10 ²		
Italy (2003) [3]	ND	6-9.5	3000	10		10	20	100		15		2	500			10 ²	
France (2010) [4]	UR					15		60							4 ^o	2.5 × 10 ²	
	R					-		-							2-3 ^o	10 ⁴ -10 ⁵	
Spain (2007) [5]	UR				10	20										10 ²	0.1
	R				-	35										10 ³ -10 ⁴	0.1
Portugal (2006) [6]	UR	6.5-8.4	1000	8		60					50		575		10 ²		
	R	6.5-8.4	1000	8		60					50		575		2 × 10 ² -10 ⁴		
Australia (2000) [7]	UR	6.5-8.5			2									10			
	R	6.5-8.5			-									10 ² -10 ⁴		10 ² -10 ⁴	
Israel (1999) [8]	ND				5	10	20								10		
Tunisia (1989) [9]	ND	6.5-8.5	7000			30	30	90									≤1
Jordan (2002) [9]	UR	6-9		9	10	50	30	100	>2	45	30	30	500			10 ^{2*}	<1
	R	6-9		9	-	150	200-300	500	-	70	45	30	500			10 ³ -n.d.*	<1
Kuwait (2001) [9]	ND	6.5-8.5				15	200	100	≥2.0	35		30	0.1	4 × 10 ^{2*}	20*		<1
Oman (1993) [9]	UR	6-9	2000	10		15	15	150			50	30	400		2 × 10 ²		<1
	R	6-9	2700	10		30	20	200			50	30	400		10 ³		<1
Saudi Arabia (2000) [9]	UR	6.0-8.5			5	10	10				10		600	2.2*			1
	R	-			-	40	40				-		-	10 ^{3*}			1
China (2007) [10]	UR	5.5-8.5				60	40		≥0.5						2 × 10 ⁴		
	R	5.5-8.5				80-100	60-100		≥0.5						4 × 10 ⁴		
Mexico (1987) [11]	UR					20	20								240*		
	R					30	30								10 ^{3*}		

UR, unrestricted irrigation; R, restricted irrigation; EC, electrical conductivity; SAR, sodium absorption rate; NTU, Nephelometric Turbidity Unit; TSS, total suspended solids; BOD, biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; TNK, total nitrogen Kjeldahl; TN, total nitrogen; N-NO₃, nitrate nitrogen; P, phosphate; °log reduction value; *MPN/100 mL; n.d. no determination.

References: 1. EPA (2012); 2. WHO (2006a); 3. Decreto Ministeriale (15/2003); 4. NOR-SASP1013629A (2010); 5. Real Decreto (1620/2007); 6. Marecos do Monte (2007); 7. NWQMS (2000); 8. Arlosoroff (2007); 9. WHO (2006b); 10. Yi et al. (2011); 11. NOM-001-ECOL-1996.

Table 2

Overview of methodological approaches used to assess soil microbial community composition, structure and activity.

Main goal	Approach	Specific goal	Outcome	Methods	E.g. References
Community activity and function	Biochemical assays	Detection or quantification of specific activities	Respiratory activity	Quantification of CO ₂ evolution	[1]
			Activity of enzymes involved in biogeochemical cycles (e.g., dehydrogenase, laccase, urease, alkaline phosphatase, arylsulfatase)	Enzymatic assays	[2–5]
			Detection of compounds produced during specific microbiological transformations (e.g., nitrite for nitrifiers, ammonia for ammonifiers)	Colorimetric assays; chromatography	[6]
	Culture dependent	Quantification of specific activities	Enumeration of total cultivable metabolic groups of organisms (e.g., ammonifiers, denitrifiers, diazotrophs, autotrophs)	MPN, plating or membrane filtration techniques	[3, 7, 8]
			Profiling	MPN, (e.g. eco-BIOLOG)	[7, 9]
	Nucleic acid dependent	Quantification of specific activities (targeted)	Detection or quantification of genes involved in particular reactions (e.g., C or N cycle)	FISH-Confocal/epifluorescence microscopy; qPCR; clone libraries	[10–13]
Quantification and identification of total potential activities (non-targeted)			Functional gene microarrays (e.g. GeoChip)	[14]	
Metagenomics Metatranscriptomics			Total DNA sequencing Total mRNA (cDNA) sequencing	[12, 15–18] [19, 20]	
Community size, composition and structure	Culture dependent	Estimation of size and composition	Enumeration of total cultivable organisms or groups of organisms (e.g., total heterotrophic aerobic/anaerobic bacteria, fungi, actinomycetes)	MPN, plating or membrane filtration techniques	[3, 7, 8]
			Enumeration of total cells	Nucleic acid staining (e.g. DAPI), Confocal/epifluorescence microscopy	[3]
	Culture independent	Estimation of size	Quantification of microbial biomass (C and/or N)	Chloroform fumigation extraction	[2, 9, 18, 21]
			Small subunit rRNA gene sequence based (16S or 18S rRNA genes)	DGGE, tRFLP, microarray (e.g. PhyloChip)	[9, 11, 22]
			Phospholipid-derived fatty acids based	PFLA extraction, chromatography	[18, 23–25]
	Profiling	Assessment of taxonomic diversity and abundance of each taxa	Small subunit rRNA gene sequence based (16S or 18S rRNA genes)	Clone libraries; high throughput technologies	[12, 15–18]
Isotope labelled small subunit rRNA gene sequence based (16S or 18S rRNA genes)			Stable isotope probing assays	[26–28]	

MPN, most probable number; FISH, fluorescence in situ hybridization; DAPI, 4',6-diamidino-2-phenylindole; DGGE, denaturing gradient gel electrophoresis; tRFLP, terminal restriction fragment length polymorphism; PLFA, phospholipid-derived fatty acids.

References: 1. Cheng et al. (2013); 2. Trasar-Cepeda et al. (2008b); 3. Lopes et al. (2011); 4. Hopkins et al. (2008); 5. López-Gutiérrez et al. (2004); 6. Roger and Ladha (1992); 7. Kidd et al. (2008); 8. Lin et al. (2008); 9. Widmer et al. (2006); 10. Eickhorst and Tippkötter (2008); 11. DeAngelis et al. (2011); 12. Jones et al. (2009); 13. Henry et al. (2006); 14. He et al. (2007); 15. Hansel et al. (2008); 16. Lopes et al. (2014); 17. Rui et al. (2009); 18. Baldrian et al. (2012); 19. Shrestha et al. (2009); 20. Helbling et al. (2012); 21. Zhong and Cai (2007); 22. Warttinen et al. (2008); 23. Bai et al. (2000); 24. Bossio et al. (1998); 25. Hjort et al. (2007); 26. Radajewski et al. (2002); 27. Eickhorst et al., 2011; 28. Hori et al. (2010).

Table 3

Examples of soil physicochemical, biochemical and microbiological properties influenced by the variation of selected parameters.

Parameters	Associated effects in the soil (and environment)		References
	Physicochemical and biochemical properties	Microbiological properties	
pH	Availability of nutrients and trace metals Mineralisation of organic matter Cation exchange capacity (CEC)	Community richness and diversity	[1–6]
Organic matter	Aggregate formation and stabilisation of soil structure Water retention Content of nutrients (N, P and S) Buffer capacity Cation exchange capacity Enzymatic activity	Selection of specific populations Soil microhabitats	[2, 7–11]
Nutrients (N, P, K)	Availability of organic and inorganic contaminants Improvement of soil fertility Increase of soil organic matter Water retention Elements cycling Leaching to groundwater and risk of eutrophication of aquatic environments	Disturbance of soil microbial communities Microbial catabolic activity	[12–20]
Salinity	Soil salinisation or sodification Decrease of aggregate stability and soil structure Soil permeability and water retention Increase of soil compaction Soil pH Negative impact on soil fertility Dynamics of organic and inorganic compounds Leaching of heavy metals	Soil microhabitats Community diversity and activity	[2, 9, 21–25]
Contaminants	Soil toxicity, terraccumulation, leaching Negative impact on soil fertility Potential direct or indirect contamination of the food chain	Community structure and diversity Increase of microbial tolerance to contaminants and/or biodegradation Spread of antibiotic resistance	[26–35]
	<i>Examples of specific effects:</i>		
	Metals:		[10, 11]
	- Enhance effects of antibiotics		[12]
	Boron:		
	- Plant toxicity		[13]
	Surfactants:		
	- Water repellency		[14]
	Antibiotics:		
	- Contribute for antibiotic resistance		[15]
	Endocrine-disrupting products and other pharmaceutical products:		
	- Interfere with normal functioning of hormone systems in wildlife		
	- Influence plants development		

References: 1. Fierer and Jackson (2006); 2. Lauber et al. (2009); 3. Rousk et al. (2010); 4. White and Greenwood (2013); 5. Sparks (2003); 6. Bloom (2000); 7. Torsvik and Ovreas (2002); 8. Sun et al. (2014); 9. Smit et al. (2001); 10. Baldock and Nelson (2000); 11. Van-Camp et al. (2004); 12. DeForest et al. (2004); 13. Kuramae et al. (2011); 14. Ramirez et al. (2012); 15. Habteselassie et al. (2013); 16. Turlapati et al. (2013); 17. Knobeloch et al. (2000); 18. Zörb et al. (2014); 19. Haynes and Naidu (1998); 20. Wu (1999); 21. Ayers and Westcot (1994); 22. Sarig et al. (1993); 23. Rietz and Haynes (2003); 24. Wong et al. (2008); 25. Ke et al. (2013); 26. Correa et al. (2010); 27. Ding et al. (2012); 28. Müller et al. (2002); 29. Sánchez-Peinado et al. (2010); 30. Bååth (1989); 31. DeRito et al. (2005); 32. Chee-Sanford et al. (2009); 33. Rooklidge (2004); 34. Swartjes (2011); 35. Parks and Edwards (2005).

Table 4

Observed effects of wastewater (WW) irrigation on soil properties.

WW	Soil description*/ culture/period of irrigation (years)/country	Effects on physicochemical parameters					Effects on microbiological parameters			Reported implications formicrobiota and/or plant production	Reference
		Organic matter pools	pH	Salinity	Metals	Others	Biomass	Enzyme activity	Others		
U, la	Vertic Xerocrept (USDA)/ citrus orchard/15/Italy	ON				NH ₄ -N, NO ₃ -N		AP, HD		Increment of available nutrients improves metabolic efficiency of soil microbiota.	[1]
U, st	Calcisols (WRB)/alfalfa, maize, barley, oats/>20/ Spain	OC				AvP		AP, BG		Increment of available P and water-soluble organic carbon is related with the increases of soil microbial biomass and activity.	[2]
U	Vertisols (WRB)/cereals and vegetables/< 80/Mexico	OC			Pb, Cd, Cu, Zn			DH	Denitrification activity Adenylate energy charge ratios	Increment of available decomposable organic material increases soil microbial biomass and activity.	[3]
U	Typic Haplustand (USDA)/ hazel orchard/20/Italy	OC, TN							Diversity of the ammonia-oxidizing bacteria	Wastewater flooding leads to the functional uniformisation of soil microbiota.	[4]
U, st	Xerorthent (USDA)/ orange-tree orchard/43/ Spain	OC				AvP		AP, BG, DH, PR, UR	Diversity of arbuscular mycorrhizal fungi	Reduction of the arbuscular mycorrhizal fungi diversity but crop vitality and productivity is not affected.	[5]
U	NR/cereals, millets, vegetable and fodder crops/5/India	OC			Fe					Metals may be accumulated by plants.	[6]
	NR/cereals, millets, vegetable and fodder crops/10/India	OC, TN			Zn, Fe, Ni, Pb						
	NR/cereals, millets, vegetable and fodder crops/20/India	OC			Zn, Cu, Fe, Ni, Pb Mn						
U, st	Loamy fine sand texture/ alfalfa hay, sudan grass and winter grains/3, 8, 20 /USA	OM			Cr, Cu, Ni, Zn					Metals may be accumulated by plants and may also be leached.	[7]
U, st	Fine clay and silt loam texture/corn/NR/China	OC, TN				AvP				Organic inputs of wastewater increases the total organic C and total N in soils.	[8]

U, st	Fine texture/forage crops /2, 5, 10/Jordan	OM, TN			Cu, Pb, Cd	AvP, K				Monitorisation of soil and plant is needed for a safe use of wastewater.	[9]
U, st	NR/persimmon orchard/4 /Israel	OC				NO ₃ -N		DH, HD	Bacterial abundance Denitrification Abundance and diversity of actinobacteria	Soil bacterial composition and function are affected by wastewater irrigation.	[10,11]
U, st	NR/orange grove/4, 8, 16, 26/Tunisia	OC			Ni, Cr, Co				Microbial abundance	Modification of the genetic structure of soil bacterial and fungal communities.	[12]
U	Xerorthent (USDA)/grape crop/20/Spain	OC				CEC, WR, AvP AgSt		AP, UR BG		Modification of soil quality indices	[13]
	Xerofluvent (USDA)/ "green filter"/20/Spain	OC				CEC, AgSt, AvP WR		AP, BG, UR			
	Xerorthent (USDA)/ orange-tree orchard/40/ Spain	OC				AvP CEC, WR, AgSt		AP BG, UR			
	Xerofluvent (USDA)/ grape crop/2/Spain	OC				AvP CEC, WR, AgSt		AP, BG, UR			
Sy	Sandy loam texture/ Mangrove swamp/0.25/China	TN			Cu, Zn, Cd, Mn	NH ₄ -N, NO ₃ -N, AvP		AP, DH	Aerobic and anaerobic bacteria Ammonia- and nitrite-oxidizing bacteria	Increment of available nutrients stimulates microbial growth and activity.	[14]

U	Leptosols (WRB)/cereals and vegetables/< -80/ Mexico	OC		Pb, Cd, Cu, Zn			DH	Denitrification activity Adenylate energy charge ratios	Increment of available nutrients and easily decomposable organic material increases soil microbial biomass and activity.	[3]
U, st	Vertic Xerofluvent (USDA)/maize/0.25/Turkey	OC					AS, AP, DH, UR		Short-term irrigation affects oil enzymatic activities.	[15]
I	Chromasols and Tenosols (ASC)/grazed pastoral/>60 /Australia	OC, TN			C/N ratio AvP, Ex Na, K			Metabolic quotient	The community structure and catabolic capability of irrigated soil are resilient.	[16]
U, tt	Horticultural soil/NR/1/ France	OM					Laccase, cellulase, PR, UR	Functional diversity of soil microorganisms	Increase of soil enzymatic activity is involved in the degradation of the organic matter brought by waste water.	[17]
U	Typic Haplustox (USDA)/ sugarcane/> 1/Brazil	OC, TN			NO ₃ -N				Wastewater irrigation increases the crop productivity.	[18]
U	Eutric Arenosol (WRB)/ lettuce/15/Senegal	OC, TN			NH ₄ -N, NO ₃ -N		HD	Bacterial community structure Ammonia - oxidizing	Ammonia-oxidizing bacterial community is stimulated by wastewater supply.	[19]
I	Loamy texture/Fodder, cereals/NR/Pakistan	OM		Zn, Cu, Ni, Cr	Ex SO ₄ , NO ₃ -N, AvP			Heavy metal resistant bacteria Vesicular arbuscular mycorrhizae	Metal intrinsic endurance of bacterial and vesicular arbuscular mycorrhizae populations is enhanced.	[20]
U, st	Argosols and Cambosols (CST)/cereals and vegetables/> -40/China			Cd, Cr, Cu, Ni, Pb, Zn	HA				Metals may be accumulated by plants.	[21]
U	NR/barley, corn, cotton, alfalfa, sorghum/80/USA			Zn	SoilCom AvP Mg				Crop production is not affected.	[22]

U	Silty sand texture/perennial ryegrass/3/Spain							Microbial abundance (total aerobic bacteria)	Low risk of microbial aquifer contamination	[23]
U	Quartzarenic Neosol (SiBCS)/eucalyptus/5/Brazil				Ex Na				Crop productivity increases	[24]
I, sy	NR/mangrove/0.5/China				Cd, Cr, Cu, Ni, Zn		AP		Heavy metals reduce plant production and decrease alkaline AP activity.	[25]
I	Rhizosphere soil/wheat/~10/India				Fe, Cr, Zn, Pb, Ni, Cd, Cu			Metal resistant <i>Azotobacter chroococcum</i> isolates	Wastewater leads to an increase of metal resistance in rhizospheric <i>A. chroococcum</i> .	[26]
U	Mollic Leptosol and Eutric Vertisol (WRB)/maize/5, 90/Mexico				Cr, Cu, Ni, Zn, Pb	AvP		Arbuscular mycorrhizal fungi free spores	Heavy metal content and arbuscular mycorrhizal fungi free spores were negatively correlated.	[27]
I	NR/agricultural/12/India				Fe, Ni, Zn			Microbial abundance and diversity	The microbiological properties of the soil in the long-term exposure were not affected.	[28]
U, st	Eutric Histosol (WRB)/different grasses/3/Poland					WC; Eh	UR		Effects of wastewater on enzyme activities are dependent of plant type.	[29]
							AP, DH			
	WC									
	UR									
Eutric Histosol (WRB)/salix/3/Poland	Eh	AP	AP, DH							
U	NR/Parks/NR/China					AB, DP		Diversity and abundance of ARG and integrase genes	Possible propagation of antibiotic resistance	[30]
U; I	Silty clay loam texture/crops/50/China					ED, Ph			Irrigation could lead to the accumulation of some emerging contaminants.	[31]