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Effects of 18 pharmaceuticals on the physiological diversity of edaphic microorganisms

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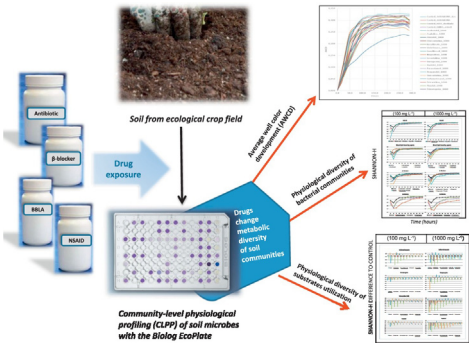
HIGHLIGHTS

- Toxicity of 18 pharmaceuticals on soil microbial communities was measured.
- Drugs change the diversity patterns of carbon substrate utilization of microbials.
- Antibiotics and nadolol imposed the greatest impact on microbial communities.
- Drug's physicochemical properties determine metabolic diversity of soil communities.

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceutical residues can enter the terrestrial environment through the application of recycled water and contaminated biosolids to agricultural soils, were edaphic microfauna can would be threatened. This study thus assessed the effect of 18 widely consumed pharmaceuticals, belonging to four groups: antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), blood lipid-lowering agents (BLLA) and β-blockers, on the physiology of soil microbial communities from a ecological crop field. Biolog EcoPlates, containing 31 of the most common carbon sources found in forest and crop soils, were used to calculate both the averaged well colour development (AWCD), as an indicator of the entire capacity of degrading carbon sources, and the diversity of carbon source utilization, as an indicator of the physiological diversity. The results show that pharmaceuticals impact microbial communities by changing the ability of microbes to metabolize different carbon sources, thus affecting the metabolic diversity of the soil community. The toxicity of the pharmaceuticals was inversely related to the log K_{ow}; indeed, NSAIDs were the least toxic and antibiotics were the most toxic, while BLLA and β-blockers presented intermediate toxicity. The antibiotic sulfamethoxazole imposed the greatest impact on microbial communities at concentrations from 100 mg/L, followed by the other two antibiotics (trimethoprim and tetracycline) and the β-blocker nadolol. Other chemical parameters (i.e. melting point, molecular weight, pK_a or solubility) had little influence on toxicity. Microbial communities exposed to pharmaceuticals having similar physicochemical characteristics presented similar physiological diversity patterns of carbon substrate utilization. These results suggest that the repeated amendment of agricultural soils with biosolids or sludges containing pharmaceutical residuals may result in soil concentrations of concern regarding key ecological functions (i.e. the carbon cycle).

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1. Introduction

Pharmaceutical compounds such as non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics are used extensively worldwide and their consumption, predominantly in developed countries is assumed to be greater than several hundred tons per year (de Garcia et al., 2013; Wise, 2002). Recent studies estimate that in 2011 about two million tonnes of pharmaceuticals were produced only in China (Liu and Wong, 2013).

Approximately 70% of administered drugs are estimated to be released unchanged into the environment (Kummerer and Henninger, 2003), which would explain the increasing ubiquity of pharmaceuticals in the environment. Pharmaceuticals deserve special attention as environmental contaminants because they are designed to interfere with biological processes at low concentrations through a specific mode of action and to persist in the animal or human body. Although, once in the environment, they are generally less persistent than classical persistent organic pollutants (POPs), the fact is that many drugs are not readily degraded in the environment and their continuous release makes them persistent o semi-persistent (Kasprzyk-Hordern et al., 2008; Khetan and Collins, 2007).

Pharmaceuticals are introduced into the environment via a number of routes, but untreated and treated sewage are the primary sources (Ternes et al., 2004; Yu et al., 2011). The wastewater treatment process does not fully eliminate pharmaceuticals from the final effluent, and thus these compounds can accumulate in sewage sludge or biosolids (Martin et al., 2012a; Miege et al., 2009). Therefore, pharmaceutical residues can enter the terrestrial environment following soil applications of wastewater (recycled water) and through the application of contaminated biosolids to agricultural soils (Chen et al., 2013; Kinney et al., 2006; Xia et al., 2005). The available data show levels of human pharmaceuticals in biosolids and wastewater in the range of ng/kg to μg/kg. For example, some authors have detected diclofenac, ibuprofen or paracetamol in wastewater at values ranging from 0.2 μg/L to 246 μg/L (Gomez et al., 2007; Lishman et al., 2006; Roberts and Thomas, 2006; Rosal et al., 2010; Stuelten et al., 2008; Ternes, 1998). Salicylic acid has been found at average concentrations of 0.106 μg/L (Lishman et al., 2006), in primary sludge (561 μg/kg dm), in digested sludge (32.9 μg/kg dm) (Martin et al., 2012a) and ibuprofen and diclofenac in sewage sludge at concentrations up to 741.1 ng/g dry weight (Martin et al., 2012a; Nieto et al., 2010; Radjenovic et al., 2009). Ibuprofen, paracetamol and salicylic acid have also been found in biosolids at concentrations from 130 μg/kg to 547 μg/kg (Albero et al., 2014; McClellan and Halden, 2010).

Regarding blood lipid-lowering agents, simvastatin has been detected in effluent concentrations, at roughly 100–300 ng/L (Ottmar et al., 2012). Gemfibrozil and lovastatin have also been detected in wastewater treatment plant effluents at concentrations below 1 μg/L (Lishman et al., 2006; Rosal et al., 2010) and at concentrations below 1 μg/L (Conley et al., 2008), respectively. No data are available for the presence of simvastatin and lovastatin in biosolids. However, traces of gemfibrozil have been found in biosolids in the ng/L range (Albero et al., 2014). Data about β-blockers in biosolids are very scarce as well. Tetracycline is the only antibiotic detected at concentrations in the milligram range in biosolids (Hamscher et al., 2002; McClellan and Halden, 2010). The amendment of soils and irrigation with pharmaceuticals occurs repeatedly so it is necessary to assess the effects of these substances on soil ecosystems.

Environmental microorganisms play a key role in fundamental ecological processes such as biogeochemical cycling and organic contaminant degradation, thus providing different ecosystem services, among others the maintenance of soil and water quality (Reed and Martiny, 2007). This is largely based on the metabolic activity of microorganisms, which may involve the degradation of contaminants. This biodegradation is considered to be the most important process for eliminating the majority of xenobiotics, including pharmaceuticals (Caracciolo et al., 2015; Carvalho et al., 2014; de Groot et al., 2002; Topp et al., 2013).

Microorganisms may also play the role of environment quality bioindicators due to their quick and sensitive reactions, even to small environmental fluctuations (Gryta et al., 2014). Microorganisms have a high surface area-to-volume ratio, because of their small size and therefore, they can provide a large contact interface, which would interact with the surrounding environment. The biological parameters as the population number, biochemical activities, and the diversity of microbial communities could be fast and largely modified by various environmental or anthropogenic factors. Therefore, soil microorganisms are suitable to act as a "biomarker" and are commonly used to evaluate the influence of chemicals on soil system (Cycon and Piotrowska-Seget, 2009).

In this regard, the occurrence of certain pharmaceuticals in the environment, especially those with acute impacts on the functioning of microorganisms, may affect key ecological soil processes.

The 18 drugs selected in this study belong to four therapeutic groups: non-steroidal anti-inflammatory drugs (NSAIDs), blood lipid-lowering agents (BLLA), β-blockers and antibiotics. Little is known about the specific behavior and concentrations of these classes of drugs in soils. These active compounds might either accumulate in soil or be absorbed by crops, or be readily available for transport into surface water and groundwater through leaching and overland flow runoff (Jongbloed and Lenis, 1998), thereby contaminating both the surrounding surface water and groundwater (Kemper, 2008; Topp et al., 2008). Some evidence suggests that some pharmaceuticals may break down depending on soil conditions, yielding degradation products (Pino et al., 2015). Previous studies have indicated that pharmaceuticals have detrimental effects on natural microbial communities and their key functions (Caracciolo et al., 2015) through the inhibition of microbial activity, alterations to soil enzymatic activity and changes in structural diversity. However, the data are still unsystematic and fragmented.

This study evaluated the effects of 18 of the most commonly used human pharmaceuticals on the soil microbial community regarding their physiological diversity and their capacity to degrade carbon sources.

The literature shows that these four drug families considered are among the most frequently detected in the environment (Nikolaou et al., 2007). In addition, drugs that have been selected in each family usually show the highest concentration values in drug identification studies in the environment (Martin et al., 2012a; Petrie et al., 2015; Santos et al., 2010) and are also among the widely consumed pharmaceuticals in the world (der Beek et al., 2016).

2. Material and methods

2.1. Chemicals

We studied four NSAIDs (ibuprofen, diclofenac, paracetamol and salicylic acid); five blood lipid-lowering agents (bezafibrate, gemfibrozil, atorvastatin, simvastatin and lovastatin); six β-blockers (propranolol, atenolol, acebutolol, metoprolol, timolol and nadolol); and three antibiotics (sulfamethoxazole, trimethoprim and tetracycline). Table 1 summarizes the relevant information of the pharmaceuticals and the chemicals and solvents used in this study and Table S1 (Supporting Information) shows the physico-chemical characteristics of these pharmaceuticals.

2.2. Experimental soils preparation

Soil was collected in July 2014 from an ecological crop field (*La Cerrada*, San Mateo de Gállego, Zaragoza, NE Spain). The physicochemical characteristics of this soil are provided in Table S2 (Supporting Information). The soil was sieved at <2 mm and kept in plastic containers (2 L volume) in a clean room. Later, 200 g dry soil were transferred into plastic containers (1 L volume) and thoroughly homogenized with pharmaceuticals at calculated concentrations in order to