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Impact of *Artemisia absinthium* hydrolate extracts with nematicidal activity on non-target soil organisms of different trophic levels

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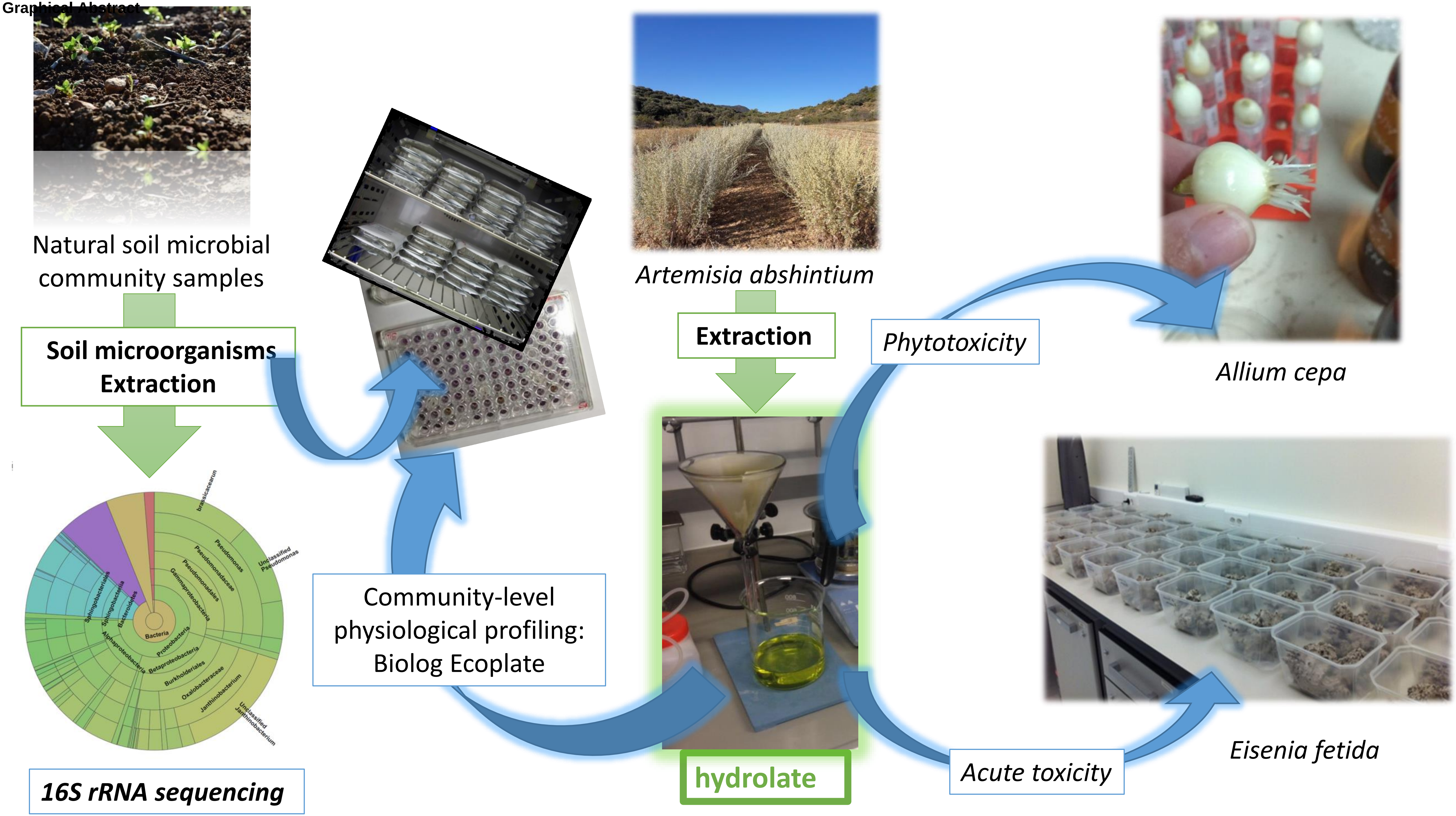
Graphical Abstract



Natural soil microbial community samples

Soil microorganisms Extraction

16S rRNA sequencing

Community-level physiological profiling: Biolog Ecoplate

Artemisia abshintium

Extraction

hydrolate

Phytotoxicity

Allium cepa

Acute toxicity

Eisenia fetida

HIGHLIGHTS

1. Soil ecotoxicity *of a new pesticide from Artemisia absinthium is evaluated*
2. The hydrolate of *A. absinthium* inhibits the growth of *Allium cepa* roots
3. The hydrolate strongly affects the survival of the earthworm *Eisenia fetida*
4. Hydrolate decreases the growth of a community of bacteria from a natural soil
5. The physiological diversity of the microbial community is only slightly affected

Impact of *Artemisia absinthium* hydrolate extracts with nematicidal activity on non-target soil organisms of different trophic levels

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Natural pesticides are considered a good alternative to synthetic pesticides to reduce environmental impacts. However, biopesticides may have unknown effects on the environment, and can affect non-target organisms. In this study, the ecotoxicological effects of an aqueous extract (hydrolate) from Spanish populations of *Artemisia absinthium* (var. Candial) showing a promising biopesticide activity, were evaluated on non-target soil organisms from different trophic levels (natural microbial communities characterized through 16S rRNA gene sequencing, the earthworm *Eisenia fetida* and the plant *Allium cepa*). The hydrolate usually was considered as a by-product of the distillation to obtain essential oils. However, recently has been found to have nematicide properties. The hydrolate caused acute toxicity at values of LC_{50} of 3.87% v/v for *A. cepa* and 0.07 mL/g for *E. fetida*. All the concentrations except for the most diluted (1% v/v) reduced the bacterial physiological activity compared to controls (LC_{50} = 25.72% v/v after 24 h of exposure). The hydrolate also slightly altered the ability of the microbial community to degrade carbon substrates. These results indicate that the hydrolate from *A. absinthium* may affect the survival and metabolic abilities of key soil organisms.

Key words: biopesticide, acute toxicity, Artemisia absinthium, soil organisms.

1. Introduction

The widespread application of synthetic pesticides has become a continous practice during the last 100 years due to the need to obtain enough food for an exponentially growing population (Liu, et al. 2015). That intensive application provoked the accumulation of a wide variety of synthetic pesticides in different ecosystems (Kelepertzis 2014, Tejada, et al. 2017). The continuous exposure to pesticides damaged non-target organisms, affected biodiversity and led to the generation of resistant varieties of pests among other consequences (Carvalho 2017, Komarek, et al. 2010, Rosner and Markowitz 2013, Shao and Zhang 2017). These problems are also associated with the use of synthetic nematicides and the generation of their degradation products, provoking undesired environmental impacts and side effects on human health (Haydock, et al. 2006, Sanchez-Moreno, et al. 2010).

Plant-based pesticides are increasingly used to replace synthetic pesticides in pest control. For example, different plant-based nematicides (bionematicides) are extracted from agricultural residues and by-products as roots, fruit skins, flowers, seeds, bark, leaves and , stems (Timper 2014). These biopesticides from plant origins seem to have advantages that make them less harmful to the environment than synthetic ones (Benelli 2015, Govindarajan and Benelli 2016, Govindarajan, et al. 2016, Pavela 2015). These advantages allowed many plant-based formulations to be described as “Generally Recognised as Safe” (GRAS) by the Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) of the USA (Kedia, et al. 2015).

Usually synthetic pesticides are based on a few or a single ingredient, but formulations from biopesticides are a mixture of compounds. This reduces the risk of pests developing resistance to the pesticide (George, et al. 2014, Jaya, et al. 2014, Miresmailli, et al. 2006). Other advantage of plant-derived pesticides is that their active ingredients are more easily degraded for environmental factors as temperature, solar radiation or enzymatic activity (El-Wakeil 2013, Varma and Dubey 1999). Therefore, these components reduce the persistence and long-term environmental impacts of these compounds.

Many plant-derived pesticides have been obtained from the Artemisia genus (Duke, et al. 1988).

Artemisia absinthium L (wormwood) is one of the most widely distributed species of the Artemisia genus. This plant has abundant oil-producing glands that provide its intense aromatic properties (Chiasson, et al. 2001, Mihajilov-Krstev, et al. 2014).

A new variety of Artemisia absinthium (var. Candial) was obtained from domesticated Spanish populations that showed a chemical stable composition (Bailen, et al. 2013, Gonzalez-Coloma, et al. 2012, Julio, et al. 2015, Martin, et al. 2011). The hydrodistilled and vapor pressure-extracted essential oils by-product (i.e. hydrolate*)* of this domesticated *Artemisia* populations has been described (Julio et. al 2018, submitted); it contains different water-soluble volatile compounds and large amounts of active ingredients such as acids, aldehydes and amines. The properties of the hydrolate as biopesticides of *A. absinthium* were studied and have been quite promising (Ali, et al. 2015).

For example, this hydrolate presented trypanocidal and leishmanicidal activities (Gonzalez-Coloma, et al. 2012, Martinez-Diaz, et al. 2015) as well as antifungal (Julio, et al. 2015), and strong nematocidal properties (Bailen, et al. 2013, Garcia-Rodriguez, et al. 2015, Julio, et al. 2017).

Even if a large number of studies have focused on the biological activity of plant-based pesticides on target organisms (Alarcon and Cespedes 2015, Carlsen and Fomsgaard 2008, Di Fabio, et al. 2014, Yoon, et al. 2013), studies concerning toxicological studies and their effects on non-target organisms are scarce (Chelinho, et al. 2017, Shao and Zhang 2017, Singh, et al. 2015). The recent developments on the use of natural nematicides—specifically bionematicides—raises concern about their impact on the natural soil biota (Ntalli and Caboni 2012).

Due to the almost unlimited resource of bioactive plant products, the great progress in their isolation and characterization, as well as new European legislation that encourages the development of less harmful substances (Villaverde, et al. 2016), the use of biopesticides will grow in the near future. To proceed with the registration and commercialization of the obtained products, they will need the same state and regulations as synthetic pesticides. Therefore, the potential risks of soil application on non-target organisms should be evaluated and include different trophic levels.

This study is the first attempt to characterize the soil ecotoxicity of the *A. Absinthium L* hydrolate using key model soil organisms from different trophic levels: a plant (assessing the effects on root growth of *Allium cepa L.*), a soil invertebrate (assessing the lethality on *Eisenia fetida*) and the soil microbial community (assessing the ability to degrade different carbon sources). In addition to those physiological endpoints, a taxonomic analysis of the microbial community was done which will eventually allow for a deeper understanding of the hydrolate effects on these key elements of soil ecosystems.

2. Material and methods

2.1 Extraction of the vegetal material

A population of domesticated *Artemisia absinthium* (var. Candial) was harvested in an experimental cropland (Ejea de los Caballeros, Zaragoza, Spain) in the summer of 2016 (Julio, et al. 2017). The hydrolate was produced in an experimental research plant belonging to the Aragon Regional Government (http://www.cita-aragon.es) by steam-distillation for one hour.

2.2 Ecotoxicity assay with *E. fetida*

The toxicity tests were carried out following the indications of the OECD 207 (1984) methodology in a similar way to that described before (Pino, et al. 2015). In summary, adult individuals of *Eisenia fetida were acquired from the composters of TODOVERDE* (Galicia, Spain).

All the earthworms selected were adults (above 60 days of age) with clitellum and similar size (weights ranging from 300–600 mg). Before testing, earthworms were acclimatized over 15 days in a sphagnum peat conditioned substrate from the Spanish FLOWER company (Tarragona, Spain). They were kept at a stable condition: 18–25°C, pH 7.5–8 and 80–85% humidity.

The toxicity tests were carried out in polypropylene containers of 1 liter capacity with lid. Small holes were made in the lid that allowed ventilation but reduced moisture loss.

The soil for the tests was prepared with the following mixture, according to the OECD 207: industrial fine sand (Imerys Ceramics España, S.A., Spain), sphagnum peat (Verdecora vivarium, Spain), and kaolin clay (Imerys Ceramics España, S.A., Spain) in a 7:1:2 ratio. 500 g was poured in each of the test containers. The moisture of the substrate was adjusted with deionized water in an amount equivalent to 35% of the dry weight of soil and pH was adjusted to 6.0 ±0.5.

Finally, 10 adult earthworms were added to each testing jar (x 3 replicates). Hydrolate was diluted in distilled water at the following dilutions: 0.005-0.01-0.05-0.1-0.3 (mL/mg). Negative controls were prepared using the same procedure without the hydrolate.

The containers were kept under controlled environmental conditions: 20 ± 2°C, 80–85% relative humidity and 400–800 lx of light. Mortality of earthworms was measured after 14 days of treatment.The LC50 values were calculated using log-Probit analysis (Bliss 1934).

2.3 Allium cepa assay

Acute toxicity experiments with *A. cepa* were conducted according to Fiskesjo, 1993. Bulbs of *A. cepa* (var Stuttgarter Riesen de 14/21) were acquired from Fitoagrícola Company (Spain). Young onion bulbs were placed in 15 mL tubes using mineral water (VERI, Aguas de San Martín de Veri S.A., Spain) as the growth medium because of its adequate content in Ca+Mg (https://www.veri.es/es/el-producto). Ecotoxicological tests were performed using 6 replicates for each concentration: negative control-0.1-1-10-50 (% v/v) and incubating the bulbs in a dark chamber at 20°C over 72 hours. The test solutions were renewed every 24 hours.

2.4 Biolog EcoPlate™tests

2.4.1 Soil samples

The soil was obtained from an experimental crop field free of pesticides or other contaminants (CSIC, Montañana, Zaragoza, NE-Spain). The samples were collected on October 30, 2017 and taken to the laboratory in less than 15 minutes.

The soil was sieved at < 2 mm and stored in polypropilene jars with 2 L volumes at 4°C until use. The texture of the soil was: 37.3% sand; 24.7% silt and 38.0% clay; the content of organic matter was 3.86% and the pH was between 7.6 and 8.

2.4.2 Genetic study of soil microorganisms

Soil microbes were extracted by mixing 10 g of soil with 95 mL of sterilized Milli-Q water in 100 mL Erlenmeyer flasks; this method has been developed in previous studies (Muniz, et al. 2014, Pino-Otin, et al. 2017, Tiquia 2010). The soil suspension was magnetically stirred for 30 min, followed by 1 h of rest. Afterward, 10 mL of the soil suspension was transferred into 50 mL Falcon tubes and—after 1 min of sonication—the tubes were centrifuged (1000 g × 10 min). Then, 9.5 mL of the supernatant were separated and the remaining was resuspended by adding 9.5 mL of water. After repeating the above protocol five times, 47.5 mL of soil lixiviate were obtained from each sample. The lixiviates were finally centrifuged at 5000 g and stored at -80°C.

Genetic sequencing was done as described in (Pino-Otin, et al. 2019) at the at Genomics Unit Cantoblanco, Science Park, Madrid (Spain). Briefly, bacterial DNA was digested using RNAse and proteinase K enzymes and extracted using G-spin^{TM} columns (INTRON Biotechnology). Amplification of the V3–V4 region of the 16 S rRNA gene was carry out according to the technique of amplicon sequencing (Caporaso, et al. 2012, Caporaso, et al. 2011). Bioanalyzer 2100 (Agilent) was used to analyse Individual amplicon libraries. An Illumina MiSeq Instrument served to sequencing DNA samples under a 2x300 protocol. Finally, metagenomics 16S, were filtered qualitatively and mapped (see Pino-Otín, 2019 for more details).

A high percentage of taxa within each level of organization was successfully sequenced (above 90%) except at the genus (82.79%) and species (26.36%) levels (Support information SI2). The relative abundance of the highest 8 taxonomic classifications at each level are shown in Figure 3. The complete taxonomic classification is provided in Support information SI1. The sequencing is based on 209.275 total reads whose 94.0% passed the quality filtering.

2.4.3 Sample exposition to hydrolate and Biolog EcoPlate™ assay

The metabolic performance of microbial communities was assessed as the ability to degrade different carbon sources after hydrolate exposure, using Biolog EcoPlate™. This approach is widely used for characterizing the metabolic abilities –substrate preference- of microbial communities (Yu, et al. 2012). That method allows for comparing the toxicity of different compounds on microbial communities (Muniz, et al. 2014, Pino-Otin, et al. 2017, Tiquia 2010).

Biolog EcoPlates presents wells with 31 different carbon sources and tetrazolium violet, whose reduction indicates the ability of microbes to degrade every carbon sorce (Pohland 2009).

Soil lixiviate (47.5 mL) containing microbes was extracted and conserved as detailed in section 2.4.2. Mineral soil particles were removed by centrifugation (500g × 2 min). A volume of 150 µL of dilutions of *A. absinthium L* hydrolate (100%-50%-25% -10% and 1% v/v in distilled water) were deposited in the wells of the Biolog plates (1 per concentration), and 150 µL of the lixiviate containing the soil microorganisms were added. All manipulations were performed under conditions of sterility in a flow chamber. Plates were incubated 7 days at 25ºC ijn darkness and sterile conditions. The final dilution pH ranged between 7.71–8.06. The optical density (OD at 590 nm) of all Biolog plate wells was measured at time 0 (just after the incubation) and every 24 h using a Microplate Reader (model Anthos 2010) and the data was adquired and processed using ADAP 2.0 software (Biochrom, Ltd. Cambridge Science Park, Cambridge, England).

2.5 Statistics and graphical representation

Experimental data for *E. fetida* and *A. cepa* was adjusted to dose-response curves using XLSTAT Soft. (2014.5.03). Thus, the EC_{50} (effect concentration) or LC_{50} (lethal concentration) values and standard errors (SE) were calculated. Chi-square test was used to evaluate the significance of the models.

Results from Biolog EcoPlate™ assays, was represented as the average well colour development -AWCD- (see detailed method in Muniz, et al. 2014) as follows:

$$AWCD = \sum_{i=0}^{i=12} \left(OD_{t=x_i} - OD_{t=x_0} \right) \qquad [1]$$

where OD_i is the well optical density at time *i*,and $OD_{t=x0}$ that at time 0. Values of ODs at AWCD plateau were used to calculate the physiological diversity, using Paleontological Statistics Software v. 3.0 (Fang, et al. 2009, Pino-Otin, et al. 2017, Tortella, et al. 2013) as follows:

- Shannon-Weaver index (as a measure of richness):

$$H = -\sum_{i=1}^{i=12} p_i \; log_2 \; p_i \qquad [2]$$

where p_i is the ratio of absorbance of a particular well to the sum of absorbances of all microplate wells. The Shannon index, as metabolic diversity proxy, was computed considering each carbon source as a different species, and its color intensity, as its abundance. Student's t-test were used to compare samples, using XLSTAT Soft. (2014.5.03).

3. Results

3.1 Mortality of A. absinthium L hydrolate on E. fetida

Dose-response curve of *E. fetida* after 14 days of exposure to *A. absinthium L hydrolate is shown in Figure 1.* Significance was evaluated with the chi-square test and all values were very significant (P < 0.0001). The measure effect is the mortality of the earthworm.

As can be seen, the hydrolate cause a great impact on the survival of *E.fetida* with an LC_{10} = 0.02 mL/g (s.e. interval of 0.008–0.03) and an LC_{50} value of 0.07 mL/g (s.e. interval of 0.05–0.10) of hydrolate dilution.

3.2 Effects of A. absinthium L hydrolate on A. cepa

After 72 h of exposure, the hydrolate strongly inhibited the growth of the roots, yielding an LC_{10} value of 0.18% v/v (s.e. interval of 0.13–0.23%) and an LC_{50} value of 3.87% v/v (s.e. interval of 3.30–4.53%) of the dilution (see Figure 2). All values were highly significant (P < 0.0001).

3.3 Impact on diversity and physiology of soil bacteria

3.3.1 Genetic analysis of microbial populations

The highest 8 taxonomic classifications at each level and their relative abundance can be seen in Figure 3. All taxa found in the samples as well as their abundance can be consulted in Support information SI1.

Sequencing comes from 209,275 total reads and 196,682 reads passed filter of quality (94.0%).

Each level of organization was successfully sequenced (above 90% of taxa). A little less in the case of genus and species (96.91% and 41.00%, respectively) (Support information SI2).

The charts in Figure 4 show the % of taxa abundance for different taxonomic levels. The predominant phylum (Figure 3) was Proteobacteria (76.06%), then Bacteroidetes (11.29%) and Firmicutes (4.86%). Proteobacteria were the dominant phylum of non-contaminated soils (Madigan et al., 2015; Janssen 2006, Spain, et al. 2009). Bacteroidetes were also abundant groups; Firmicutes somewhat less so (Madigan et al., 2015). However, in our samples, they appear practically in the same proportion.

The diversity of Bacteroidetes seems to be a frequent feature in uncontaminated soils (Madigan et al., 2015). It is also common to find a significant proportion of unclassified microorganisms' phylotypes or minority bacterial members (Madigan et al., 2015) due to the high typical bacterial diversity of edaphic ecosystems (Spain, et al. 2009).

Among Proteobacteria, the most abundant were the Gammabacteria class (39.21% of Proteobacteria, 29.83% of the total taxa), followed by Beta (33.62% of proteobacteria, 25.57% of the total taxa) and Alphaproteobacteria class (18.07% of Proteobacteria, 13.75% of the total taxa). Although Alphaproteobacteria is usually the most frequent family of soil Proteobacteria (Janssen 2006, Spain, et al. 2009), the proportions between these three families may vary depending on the characteristics of the soil (Faoro, et al. 2010). Pseudomonadales order was the most abundant among the Gammaproteobacteria class (91.27% of Gammaproteobacterias; 27.22% of the total taxa), and all of them were of the Pseudomonas genus.

Betaproteobacteria have a wide range of ecological and metabolic characteristics (Madigan et al., 2015). Almost all the Betaprotebacteria identified were of the order Burkholderiales (91.92% of Betaproteobacteria and 23.50% of total taxa). This order is also frequently comprised of taxa of the soil and water that intervene in the carbon and nitrogen cycles (Madigan et al., 2015). Among Burkholderiales, the most abundant family was Oxalabateriaceae (81.55% of Burkholderiales and

19.17% of the total) followed by the Comamonadaceae family (17.79% of Burkholderiales and 4.18% of total taxa). The main genus of Oxalabateriaceae was Janthinobacterium (86.85% of this family and 16.65% of the total).

Members from the Oxalobacteraceae family are commonly found in the soil and rhizosphere (Aleklett, et al. 2015, Green, et al. 2007), including the genus Janthinobacterium (Scheublin, et al. 2010). The high presence of these groups in our samples could be due to the inclusion of epiphytic members of the root microbiota, which is where bacteria associated with water films and soil particles of the root surface would be expected.

The Alphaproteobacteria class showed greater diversity than the Gamma and Betaproteobacterias. This class, with almost 1000 species described, includes most of the oligotrophic bacteria capable of living in an environment that offers very low levels of nutrients.

The order Sphingomonadales in the sample (41.32% of the Alphaproteobacteria of the sample, 5.68% of the total taxa) was the most abundant, following by the order Rhizobiales (39.78% of Alphaproteobacteria; 5.47 % of the total taxa) and the order Caulobacterales (15.98% of this class; 2.20% of the total). Sphingomonas (63.62% of the Sphingomonadaceae family; 3.61% of the total) and Devosia (100% of the Hyphomicrobiaceae family and 3.92% of the total) were the genera most abundant among the order Rhizobiales.

Sphingomonas strains have been isolated from a great variety of soil environments (Ko, et al. 2017, Liu, et al. 2016)—including rhizosphere soil (Daane, et al. 2001)—and they are versatile in their nutrition and able to metabolize an extensive range of organic compounds (Madigan et al., 2015). In addition, Sphingomonas have shown unique abilities to degrade a variety of pollutants, including insecticides (Nagata, et al. 1999) and herbicides (Adkins 1999). Devosia are common bacteria from the rhizosphere (Nor, et al. 2017).

3.3.2 Average well colour development (AWCD)

Figure 5 shows the average well colour development (AWCD) of the BIOLOG plate plotted for 7 days. Points are the average of three replicates. All the hydrolate concentrations—except for the most diluted (1% v/v)—decreased the bacterial metabolism measured as AWCD compared to the control. A concentration of 10% hydrolate leads to a small decrease in the values of AWCD after

96 hours. This decrease becomes significative (Student's t-test, $P \leq 0.05$) at the 25-50 and 100% concentrations (v/v). However, the smallest concentration (1%) caused a stimulating effect on metabolism. The AWCD data at 24 hours of exposure was also represented as a dose-response curve (see Support information SI3). That allowed for the calculation of an EC_{10} value of 13.46% v/v (10.99–15.57) and an EC_{50} value of 25.72% V/V (23.29–28.24). These toxicity values were highly significant (Chi-square test, $P < 0.0001$).

3.3.3 Physiological diversity of soil microorganisms communities

Physiological diversity of soil bacterial communities was calculated from the ODs values at the plateau of the AWCD. After *A. absinthium* L hydrolates exposition, none of the doses produced significant differences in the physiological diversity of soil bacteria (Figure 6) compared to the control (Student's t-test, $P \leq 0.5$).

3.3.4 Physiological diversity of soil microorganisms substrate utilization

In order to highlight general patterns of substrate utilization, all carbon sources were avaluated grouped into 5 groups as described before (Lehman, et al. 1995, Pino-Otin, et al. 2019, Pino-Otin, et al. 2017, Weber and Legge 2009, Zak, et al. 1994). The exposure to hydrolate increased the capacity to metabolize almost all substrate classes compared to the control (Figure 7), with the exception of amines/amides that generally tended to decrease. The highest concentration of hydrolate (100%) provoked an increase in the metabolism of carbohydrates ($P = 0.03$), carboxylic and ketonic acids ($P = 0.002$) and the reduction of the metabolism of amino acids ($P = 0.005$). H-Shannon values of carboxylic and ketonic acids also increased significantly at a concentration of 50% ($P < 0.05$); carbohydrates and amino acids also increased ($P > 0.05$) to that of 25%. The exposure to hydrolate concentrations of 10% and 1% resulted in similar metabolic profiles.

4. Discussion

4.1 Effects on non-target organisms A. cepa and E. fetida

The hydrolate presented intense phytotoxicity in the case of the root grown by *A. cepa* and in the survival of the earthworm *E. fetida*.

The monoterpene (−) - (Z)-2,6-dimethylocta-5,7-diene-2,3-diol was found to be the major active component of the hydrolate of *A. absinthium*. This compound is responsible for the biopesticide activity of the hydrolate (Julio, et al. 2017).

This monoterpene has a low molecular weight of 170.25 g/mol (Wishart, et al. 2018), water solubility of 715 mg/L, pka = 20.5 and log P = 3.1 (Pino-Otin, et al. 2019). It is weakly acidic and will ionise with difficulty. All these characteristics will increase its ability to cross biological membranes and to affect biological systems. Accordingly, other monoterpenoids—including some acyclic alcohols like farnesol, citronellol or geraniol—have also been reported as bionematicides against a model nematode: *Caenorhabditis elegans* (Abdel-Rahman, et al. 2013). However, we must not lose sight of the fact that this compound, despite being the majority, is in a mixture with many others that could modify these physicochemical characteristics.

Even if bionematicides are considered safer alternatives to synthetic ones, few studies have demonstrated that these products may also affect non-target soil organisms (Chelinho, et al. 2017). These products (in this case the active molecule was naphthoquinone) affected seed germination of *Zea mays* and *Brassica napus* as well as another earthworm (*E. andrei*).

The inhibition of root growth during the *A. cepa* tests may rely on the effects at root cells. Other monoterpenes (1,8-Cineole) produced by *Sarracenia leucophylla* inhibited root growth and DNA synthesis in the apical meristem of *Brassica campestris,* indicating a potential impact on processes involved in cell proliferation (Koitabashi, et al. 1997, Nishida, et al. 2005). In addition, 1,8-Cineole was found to inhibit both proliferation and elongation of BY-2 Cultured Tobacco Cells (Yoshimura, et al. 2011). An explanation for the impacts of this compounds on cell proliferation may rely on the microtubules, a key cell organelle in cell division, that has been reported to be a target for monoterpenes (Chaimovitsh, et al. 2017).

However, the mechanism of action of (Z)-2,6-dimethyl-5,7-octadiene-2,3-diol is still unknown.

Terpenoids can cause damage to cell membranes and produce cytotoxicity (Bakkali, et al. 2008). The lipophilic property of monoterpenes (Weidenhamer, et al. 1993)—as well as the lipid oxidation and deterioration of membrane integrity and permeability in plant cells exposed to monoterpenes

(Kaur, et al. 2011, Maffei, et al. 2001, Zunino and Zygadlo 2004)—suggests that biological membranes, including mitochondrial membranes, may be the primary target of monoterpenes. It is likely the effects on biological membranes when added to the deleterious effects on mitochondrial membranes—which affects energy metabolism—disturbs a wide range of physiological and biochemical processes within the cell (Yoshimura, et al. 2011). It is clear that the wall of plant cells in the root do not prevent the action of (Z)-2,6-dimethyl-5,7-octadiene-2,3-diol on cell membranes of the roots of *A. cepa* (Yoshimura, et al. 2011).

The acute toxicity of *A. absinthium* hydrolate to *E. fetida* indicates that the product has plenty of bioavailability in the earthworm. The application of the monoterpene as a hydrolate in the soil allows an effective amount of contact with the body of the earthworm. The contaminants present in pore water are available to earthworms via dermal uptake (Vijver, et al. 2003). The biochemical composition of the earthworm's cuticle has been well investigated and is extremely tolerant to water uptake and loss (Wallwork, 1983); this potentiates an intense exchange of water across the body wall (Laverack, 1963; Saxena, et al. 2014) suggest a mechanism of action dependent upon several insecticides on *E. fetida*. One mechanism involves the binding of biocides with the outer body wall protein/lipid molecules of the earthworm, which can cause damage to the mucopolysaccharide outer layer and cause paralysis of the earthworm. Ingestion of soil particles containing the active products (Suthar, et al. 2008) or the direct uptake of active products from solid soil particles could also be another avenue of exposure to the products of the hydrolate (Vijver, et al. 2003).

4.2 Effect on microbial soil communities

Although the effects of traditional pesticides on soil microorganisms have been widely studied (Karas, et al. 2018, Oleszczuk, et al. 2014, Wang, et al. 2010), few studies can be found in the literature that analyse the effects of biopesticides of plant origin on the edaphic microbiota (Gopal, et al. 2007, Gupta, et al. 2013, Sarawaneeyaruk, et al. 2015, Singh, et al. 2015, Walvekar, et al. 2017). Usually, these studies are focused only on the culturable fraction of the bacterial population, which is only a small fraction of the total bacterial population of the edaphic ecosystem. In this study, the qualitative analysis of bacterial community structures using 16S rRNA led to the elucidation of the impact of pesticides on the total soil bacterial population.

Our results showed that dilutions above 25% v/v of the hydrolate decrease the metabolism of soil bacteria significantly. This is compatible with the effects of other plant-based biopesticides, such as the Azadirachtin (Gopal, et al. 2007, Singh, et al. 2015, Walvekar, et al. 2017) and Neem extract with Azadirachtin (Sarawaneeyaruk, et al. 2015). These biopesticides significantly reduced the abundance of the active rhizospheric bacterial population, presenting similar effects to chemical pesticides but at higher doses and less durable effects.

However, at lower concentrations (1% v/v) the hydrolate did not cause inhibition but enhanced metabolism. These stimulatory effects may be explained both by the use of biopesticides as a source of nutrients and by the elimination of bacterial competitors (e.g. fungal populations), as well as the concomitant increase in heterotrophic soil bacteria (Bending, et al. 2007, Munoz-Leoz, et al. 2011). This effect has also been reported for soil pesticides, such as Glyphosate (Ratcliff, et al. 2006) and carbendazim (Tortella, et al. 2013).

On the other hand, our results showed that hydrolate caused minor changes on the global metabolic diversity of the soil microorganisms (see Figure 6). Hight doses of hydrolate increased the microbial community capacity to metabolize some specific substrates —such as carbohydrate, carboxylic and ketonic acids and amino acids (see Figure 7) —. The small increases and decreases on the metabolism of the different substrates, compensated the AWCD values, resulting in no differences in metabolic diversity. This “smoothing effect” has been reported for other biopesticides (Walvekar, et al. 2017), and also in freshwater bacteria exposed to the same hydrolate (Pino-Otin, et al. 2019).

There is an insufficient number of studies about the toxicity mechanisms of the monoterpene (-)-(Z)-2,6-dimethylocta-5,7-diene-2,3-diol for bacteria. However, the deleterious effects of terpenoids have been related to altered cell membrane integrity and sodium channel activity that disturbs the permeability of the biological membranes of the microorganism (Bakkali, et al. 2008, Spicakova, et al. 2017). Accordingly, the prevalence of Pseudomonas in our soil samples and its resistance to chemicals based on membrane mechanisms (Tórtora et al.) may be the reason behind the small impacts detected in microbial metabolism. Pseudomonas are considered one of the more abundant taxa in soil bacterial communities (Alexander, 1977; (Janssen 2006). In addition, Pseudomonas are characterized because they can use a great diversity of organic compounds as a source of carbon and energy for growth.

In the previous study by Pino-Otin et al (2019), *Vibrio fisheri*—which are gram-negative bacteria with high sensitivity to the hydrolate—it was suggested that active compounds may cross the complex cell wall of Proteobacterias, including the outer membrane.

Probably, many of these doubts can be clarified when the mode of action of the biocidal component, 2,6-dimethylocta-5,7-diene-2,3-diol from the *A. absinthium* hydrolate be better understand.

4.3 Enviromental relevance

This hydrolate presented nematicidal activity agains a target organism *Meloidogyne javanica*, a pathogen nematode for plants, at 33% concentration (Julio, et al. 2017). Our results showed acute toxicity at lower concentrations than that needed to act as pesticide for the three non-target organisms. From lowest concentration to higher: *A cepa* (LC_{50}=3,87% v/v), *E. fetida* (values of LC_{50} are equivalent to a hydrolate dilution of 20% v/v in water applied to the 600 gr of soil) and soil bacteria in which, at 25% v/v hydrolato concentrations, a significant inhibition of growth occurs.

On the other hand, the nematicidal activity of the hydrolate decreases somewhat over time; after 28 days the hydrolate inhibits still more than 75% of Meloidogyne Javanica hatching (Julio, et al. 2017)*. This agrees with the fact the the AWCD values in bacterial test suggest a recover of the system at 7 days (see Figure 5). This results highligths the need for more detailed studies on the persistence of activity of this extract and its components to confirm that these effects may be less permanent than in the case of traditional pesticides.*

Conclusion

A. absinthium hydrolate caused acute toxicity for non-target organisms belonging to different trophic levels. The toxicity can be detected at low hydrolate concentrations and in a dose-dependent manner. The hydrolate caused phytotoxicity in *Allium cepa,* leading to a strong inhibition in root growth; it also caused high mortality in the earthworm *Eisenia fetida*. The hydrolate also reduced the bacterial metabolism of a natural soil microbial community, although the physiologic diversity the community analysed through 16S rRNA gene sequencing was only slightly modified. These toxicity effects occur in concentrations lower than those required to control the target organisms. The physical and chemical properties of this main component ((-) -(Z) -2,6-dimethylocta-5,7-diene-2,3-diol), probably make it possible for it to cross biological membranes, which would explain such an intense effect on such diverse soil organisms. These results show that plant-based biopesticides are not completely safe for the soil, thus highlighting the need for more studies in natural soils over longer times to ensure that these compounds are safer alternatives to synthetic pesticides.

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Figure

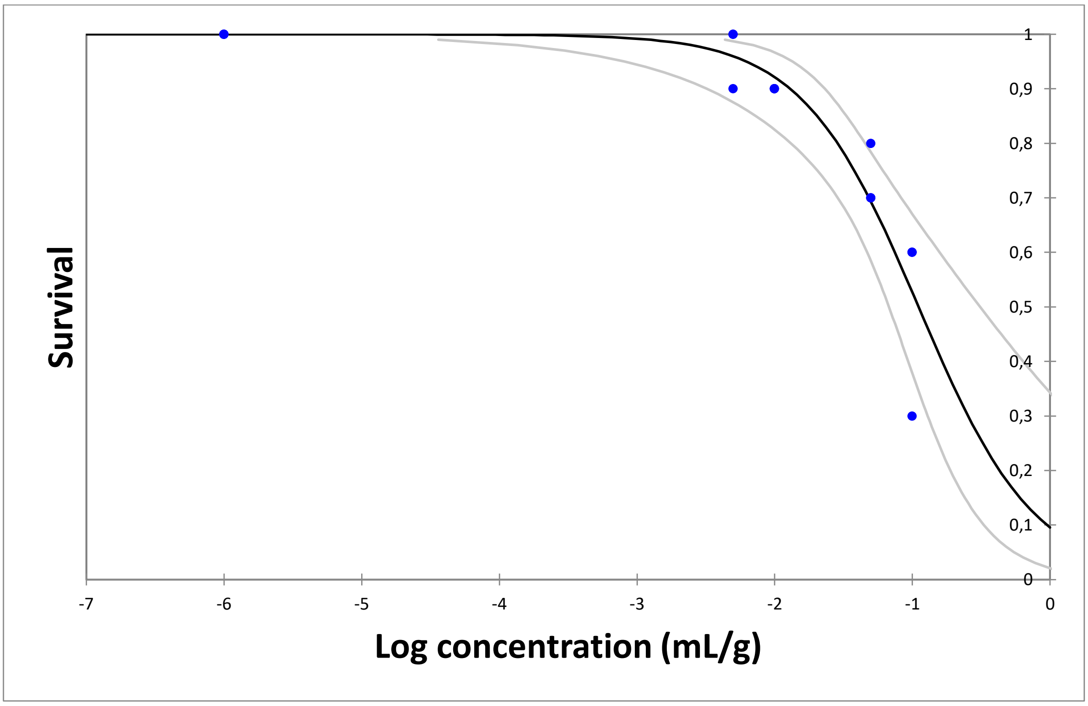

Figure 1. The black curve shows the dose-response of *Eisenia fetida* after exposure to *Artemisia absinthium L* hydrolate during *14 days and represents the average value of three replicates. Grey lines* are the confidence limits (95%).

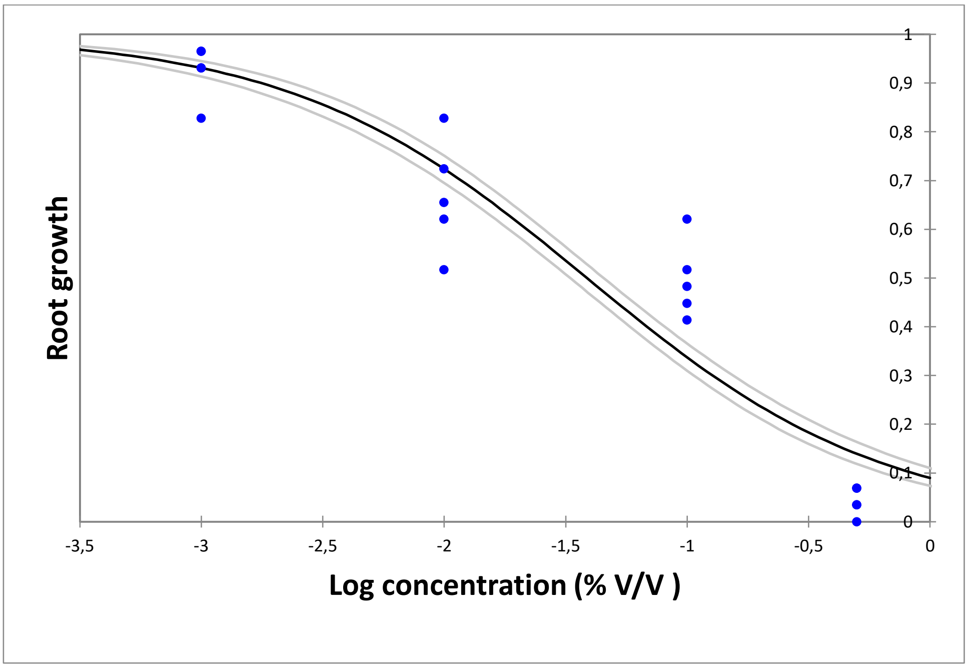

Figure 2. The curve shows the dose-response of *Allium cepa* after exposure to *A. absinthium L* hydrolate during 72 hours. As *before, grey lines* are the confidence limits (95%). Curve is the average value of six replicates. The measured effect is the inhibition of the growth of the roots.

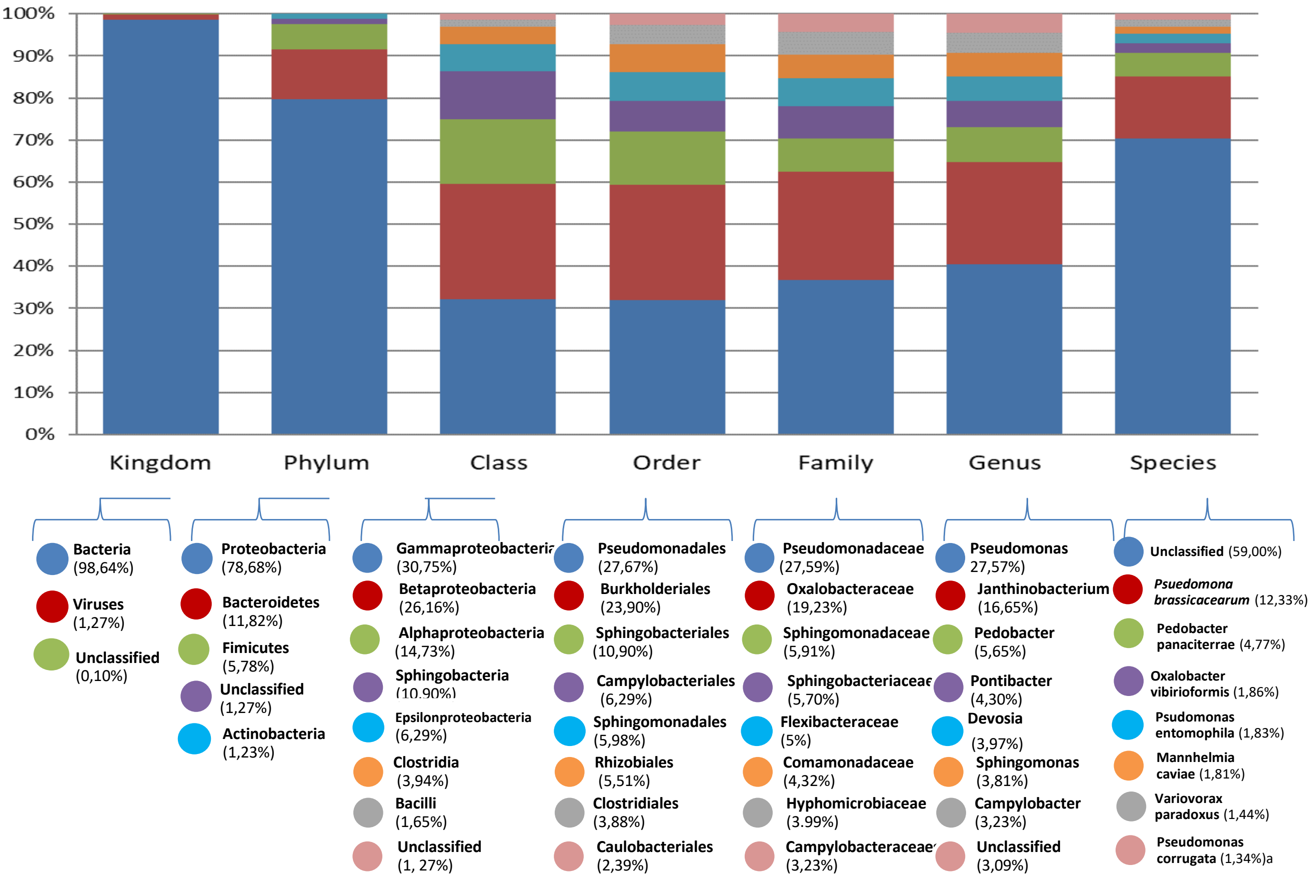

Figure 3. Eight more abundant taxa in each taxonomic level found in the samples obtained from an experimental field (Zaragoza, NE Spain). The percentages indicate relative abundances within each level.

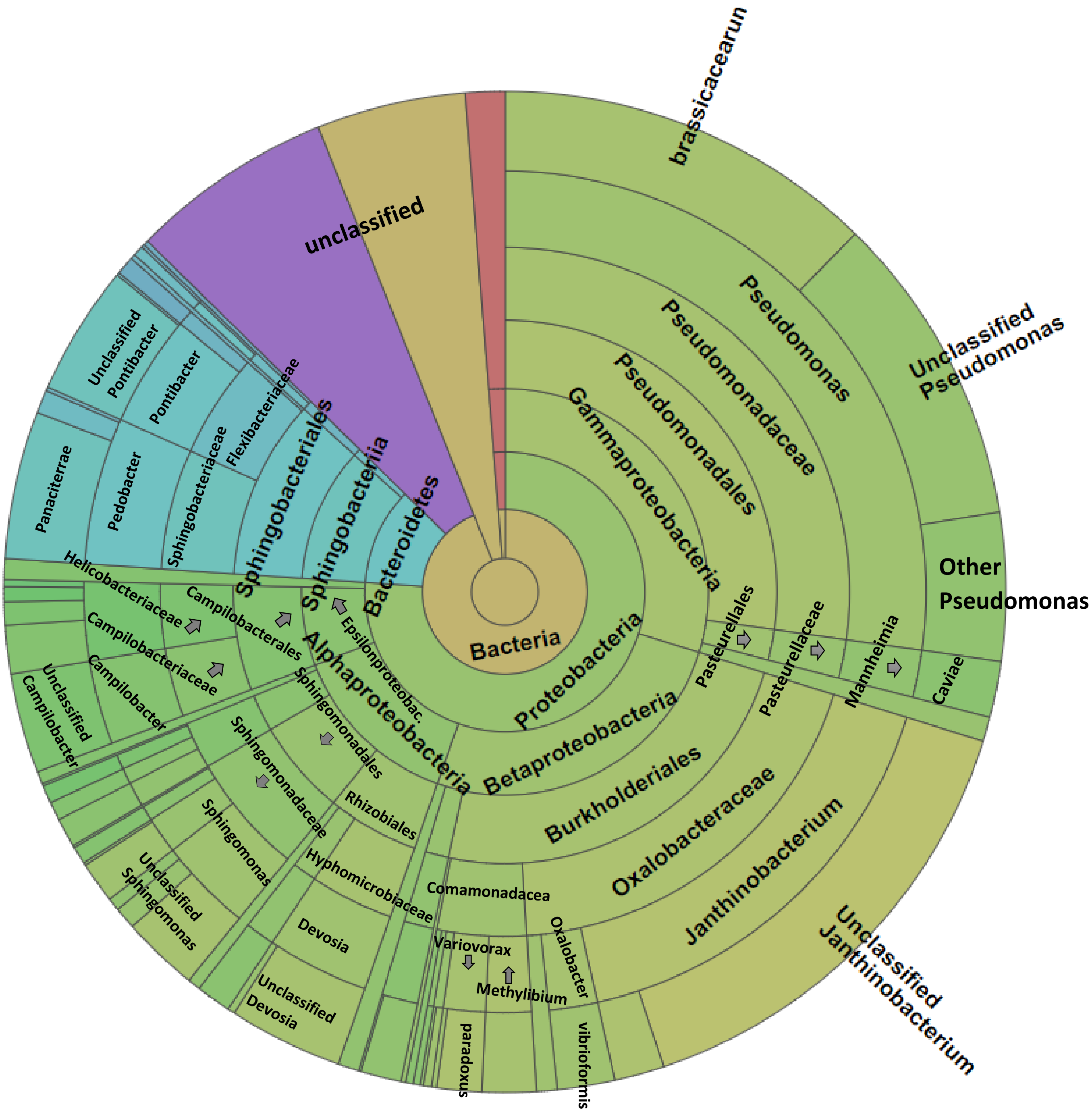

Figure 4. The graph shows the abundance of taxa for different taxonomic levels found in the samples obtained from an experimental field (Zaragoza, NE Spain). From outside the circle to inside: species, genus, family, order, class and phylum.

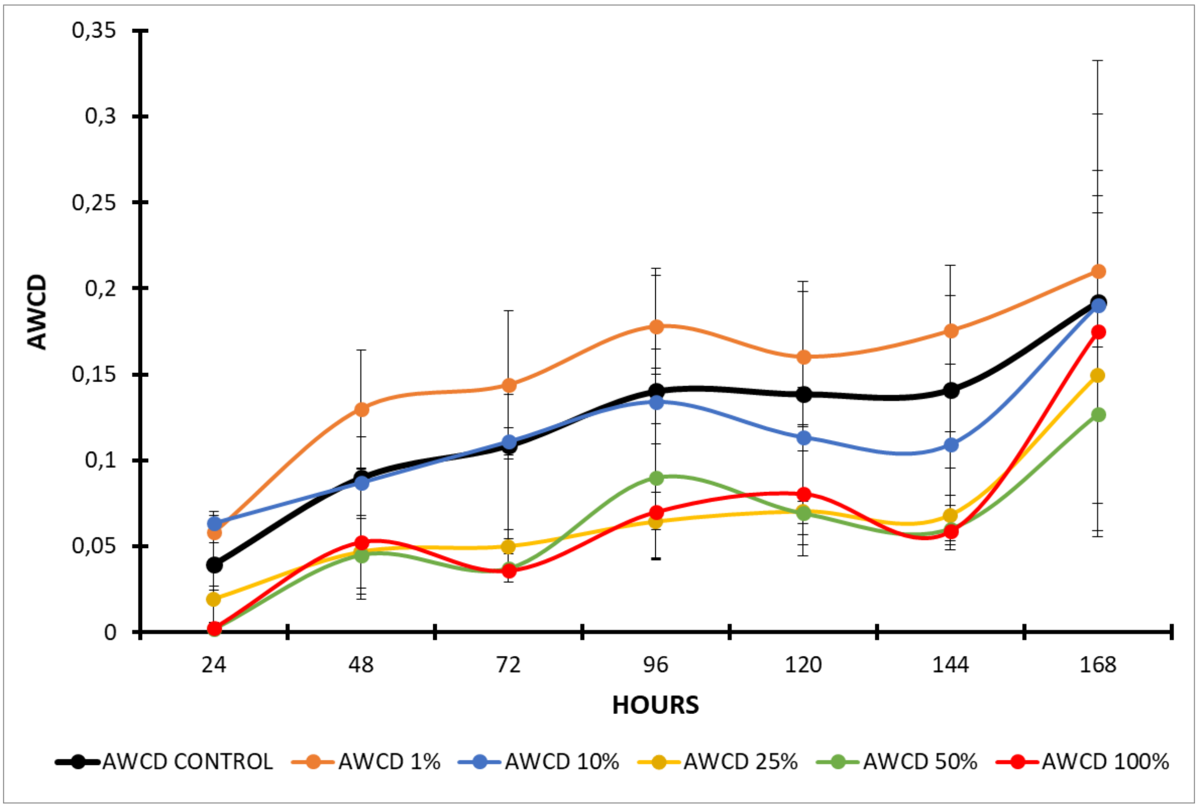

Figure 5. 168-h incubation of soil microorganisms exposed to *A. absinthium* L hydrolate concentrations (% v/v). Curves are the Average Well Color Development (AWCD) in Biolog EcoPlates of metabolized substrates. Points in curves are the average value of three replicates. Black curve is the control (bacteria only treated with water). The standard deviation of mean of the three replicates are represented by error bars.

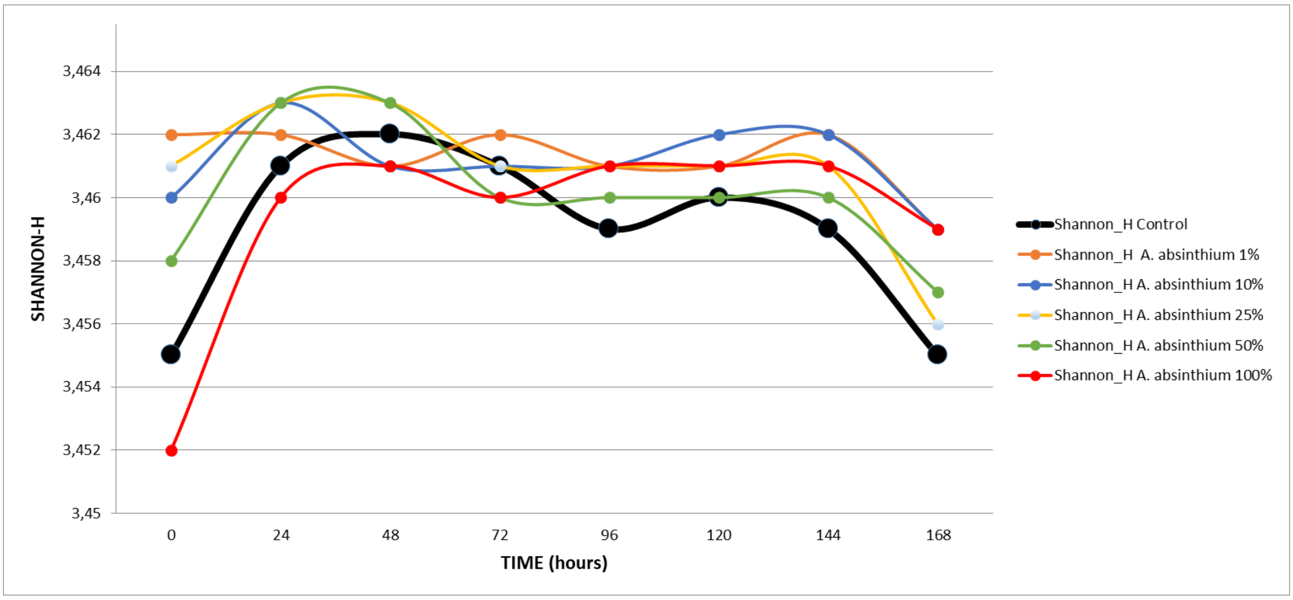

Figure 6. Curves represent the variation of the Shannon–Weaver index of soil bacteria communities treated with *A. absinthium* L hydrolate during 168 hours. Concentrations are in % V/V. Black line are the control.

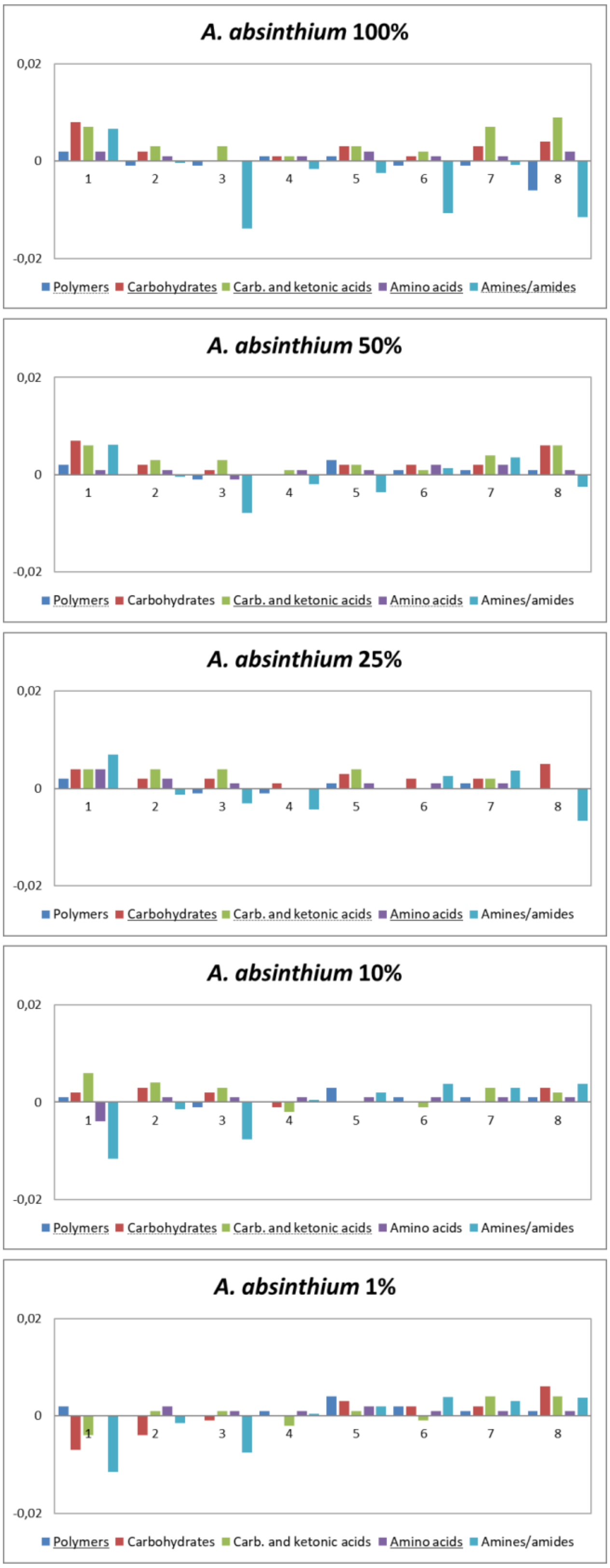

Figure 7. Bars represent the variation of the Shannon–Weaver index for each group of metabolites, of soil bacteria communities treated with *A. absinthium* L hydrolate during 168 h. Concentrations are in % v/v. Data was measured at intervals of 23 hours, 8 times, and values were obtained by subtracting those from the negative control (x-axis values). A continuous line under metabolic groups indicated that differences regarding the control are statistically significant ($p < 0.05$). Lower significance ($p < 0.5$) was indicate by a dashed lines.

S.I. 1. Complete taxonomic classification of soil microorganisms from an experimental field (Zaragoza, NE Spain).

Level	Group	Reads	Percentage
Kingdom	Unclassified	187	0.09507734
Kingdom	Bacteria	194000	9.863.638
Kingdom	Viruses	2495	1.268.545
Phylum	Unclassified	1154	0.5867339
Phylum	Proteobacteria	154754	7.868.234
Phylum	Bacteroidetes	23255	1.182.365
Phylum	Firmicutes	11369	5.780.397
Phylum	Actinobacteria	2416	1.228.379
Phylum	DNA	2495	1.268.545
Phylum	Verrucomicrobia	198	0.1006701
Phylum	Deferribacteres	207	0.105246
Phylum	Tenericutes	109	0.05541941
Phylum	Gemmatimonadetes	256	0.1301593
Phylum	Cyanobacteria	433	0.2201523
Phylum	Caldithrix	8	0.00406748
Phylum	Nitrospirae	6	0.00305061
Phylum	Chloroflexi	10	0.005084349
Phylum	Thermi	2	0.00101687
Phylum	Acidobacteria	2	0.00101687
Phylum	Thermotogae	3	0.001525305
Phylum	Spirochaetes	1	0.000508435
Phylum	Planctomycetes	1	0.000508435
Phylum	Synergistetes	1	0.000508435
Phylum	Thermodesulfobacteria	2	0.00101687
Class	Unclassified	1686	0.8572213
Class	Gammaproteobacteria	60472	3.074.608
Class	Sphingobacteriia	21438	1.089.983
Class	Betaproteobacteria	51452	2.615.999
Class	Epsilonproteobacteria	12375	6.291.883
Class	Alphaproteobacteria	28976	1.473.241
Class	Bacilli	3248	1.651.397
Class	Clostridia	7749	3.939.862
Class	Actinobacteria	2396	121.821
Class	Group II	2495	1.268.545
Class	Verrucomicrobiae	55	0.02796392
Class	Deltaproteobacteria	1139	0.5791074
Class	Deferribacteres	207	0.105246
Class	Mollicutes	109	0.05541941
Class	Gemmatimonadetes	256	0.1301593
Class	Bacteroidia	1461	0.7428234
Class	Oscillatoriophycideae	119	0.06050376
Class	Opitutae	143	0.07270619
Class	Caldithrixae	8	0.00406748

Class	Nostocophycideae	288	0.1464293
Class	Flavobacteriia	240	0.1220244
Class	Erysipelotrichi	325	0.1652414
Class	Acidimicrobiia	5	0.002542175
Class	Synechococcophycideae	3	0.001525305
Class	Nitrospira	6	0.00305061
Class	Anaerolineae	8	0.00406748
Class	Deinococci	2	0.00101687
Class	Nitriliruptoria	9	0.004575914
Class	Acidobacteria	2	0.00101687
Class	Thermotogae	3	0.001525305
Class	Spirochaetes	1	0.000508435
Class	Brocadiae	1	0.000508435
Class	Synergistia	1	0.000508435
Class	Ktedonobacteria	2	0.00101687
Class	Thermodesulfobacteria	2	0.00101687
Order	Unclassified	2706	1.375.825
Order	Sphingobacteriales	21438	1.089.983
Order	Burkholderiales	46999	2.389.593
Order	Campylobacterales	12375	6.291.883
Order	Rhizobiales	11432	5.812.428
Order	Neisseriales	43	0.0218627
Order	Lactobacillales	1572	0.7992597
Order	Pseudomonadales	54427	2.767.259
Order	Clostridiales	7639	3.883.934
Order	Sphingomonadales	11754	5.976.144
Order	Methylophilales	2855	1.451.582
Order	Actinomycetales	2381	1.210.584
Order	(Microviridae)	2495	1.268.545
Order	Pasteurellales	3658	1.859.855
Order	Rhodocyclales	1053	0.535382
Order	Enterobacteriales	470	0.2389644
Order	Rhodospirillales	965	0.4906397
Order	Verrucomicrobiales	55	0.02796392
Order	Caulobacterales	4696	238.761
Order	Desulfovibrionales	847	0.4306444
Order	Desulfuromonadales	259	0.1316846
Order	Deferribacterales	207	0.105246
Order	Acholeplasmatales	36	0.01830366
Order	Bacillales	1653	0.840443
Order	Gemmatimonadales	256	0.1301593
Order	Bacteroidales	1461	0.7428234
Order	Xanthomonadales	936	0.4758951
Order	Chroococcales	117	0.05948689
Order	Alteromonadales	94	0.04779289
Order	Coriobacteriales	82	0.04169166
Order	Entomoplasmatales	10	0.005084349
Order	Bifidobacteriales	12	0.006101219
Order	Oceanospirillales	229	0.1164316
Order	Legionellales	111	0.05643628

Order	Mycoplasmatales	63	0.0320314
Order	Rickettsiales	28	0.01423618
Order	Pelagicoccales	142	0.07219776
Order	Caldithrixales	8	0.00406748
Order	Stigonematales	280	0.1423618
Order	Flavobacteriales	240	0.1220244
Order	Erysipelotrichales	325	0.1652414
Order	Rhodobacterales	38	0.01932053
Order	Salinisphaerales	45	0.02287957
Order	Aeromonadales	14	0.007118089
Order	Kiloniellales	3	0.001525305
Order	Acidimicrobiales	5	0.002542175
Order	Pseudanabaenales	3	0.001525305
Order	Myxococcales	6	0.00305061
Order	Thiohalorhabdales	2	0.00101687
Order	Nitrospirales	6	0.00305061
Order	Gallionellales	4	0.00203374
Order	Syntrophobacterales	16	0.008134959
Order	Chromatiales	43	0.0218627
Order	Thiotrichales	21	0.01067713
Order	Caldilineales	8	0.00406748
Order	Vibrionales	1	0.000508435
Order	Turicibacterales	4	0.00203374
Order	Gemellales	4	0.00203374
Order	Natranaerobiales	3	0.001525305
Order	Thermoanaerobacterales	5	0.002542175
Order	Bdellovibrionales	3	0.001525305
Order	Deinococcales	2	0.00101687
Order	Euzebyales	9	0.004575914
Order	Halanaerobiales	1	0.000508435
Order	Acidobacteriales	2	0.00101687
Order	Thermotogales	3	0.001525305
Order	Borreliales	1	0.000508435
Order	Nostocales	8	0.00406748
Order	Brocadiales	1	0.000508435
Order	Synergistales	1	0.000508435
Order	Thermogemmatisporales	2	0.00101687
Order	Thermodesulfobacteriales	2	0.00101687
Order	Thermicanales	4	0.00203374
Order	Desulfobacterales	1	0.000508435
Order	Puniceicoccales	1	0.000508435
Order	Hydrogenophilales	1	0.000508435
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Family	Oxalobacteraceae	37830	1.923.409
Family	Neisseriaceae	43	0.0218627
Family	Streptococcaceae	1399	0.7113005
Family	Hyphomicrobiaceae	7844	3.988.164
Family	Comamonadaceae	8490	4.316.613
Family	Sphingobacteriaceae	11218	5.703.623

Family	Pseudomonadaceae	54262	275.887
Family	Ruminococcaceae	2033	1.033.648
Family	Sphingomonadaceae	11621	5.908.523
Family	Campylobacteraceae	6344	3.225.511
Family	Methylophilaceae	2855	1.451.582
Family	Helicobacteraceae	6006	305.366
Family	Rhizobiaceae	2487	1.264.478
Family	Micrococcaceae	690	0.3508201
Family	Microviridae	2495	1.268.545
Family	Pasteurellaceae	3658	1.859.855
Family	Rhodocyclaceae	1053	0.535382
Family	Lachnospiraceae	3849	1.956.966
Family	Enterobacteriaceae	470	0.2389644
Family	Rhodospirillaceae	870	0.4423384
Family	Verrucomicrobiaceae	55	0.02796392
Family	Caulobacteraceae	4696	238.761
Family	Propionibacteriaceae	278	0.1413449
Family	Desulfovibrionaceae	830	0.422001
Family	Methylobacteriaceae	416	0.2115089
Family	Chitinophagaceae	337	0.1713426
Family	Deferribacteraceae	207	0.105246
Family	Acholeplasmataceae	36	0.01830366
Family	Staphylococcaceae	1298	0.6599485
Family	Gemmatimonadaceae	256	0.1301593
Family	Clostridiaceae	1438	0.7311294
Family	Porphyromonadaceae	1011	0.5140277
Family	Xanthomonadaceae	927	0.4713192
Family	Alteromonadaceae	63	0.0320314
Family	Paenibacillaceae	249	0.1266003
Family	Coriobacteriaceae	82	0.04169166
Family	Dietziaceae	43	0.0218627
Family	Corynebacteriaceae	824	0.4189504
Family	Entomoplasmataceae	10	0.005084349
Family	Bifidobacteriaceae	12	0.006101219
Family	Bacillaceae	87	0.04423384
Family	Halomonadaceae	195	0.09914481
Family	Nocardiaceae	374	0.1901547
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Family	Enterococcaceae	3	0.001525305
Family	Oceanospirillaceae	26	0.01321931
Family	Mycoplasmataceae	63	0.0320314
Family	Bradyrhizobiaceae	67	0.03406514
Family	Rickettsiaceae	14	0.007118089
Family	Pelagicoccaceae	142	0.07219776
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Family	Burkholderiaceae	250	0.1271087
Family	Caldithrixaceae	8	0.00406748
Family	Rivulariaceae	280	0.1423618
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Family	Nocardioidaceae	21	0.01067713

Family	Alcaligenaceae	127	0.06457124
Family	Moraxellaceae	164	0.08338333
Family	Erysipelotrichaceae	306	0.1555811
Family	Odoribacteraceae	127	0.06457124
Family	Hyphomonadaceae	5	0.002542175
Family	Bartonellaceae	17	0.008643394
Family	Microbacteriaceae	9	0.004575914
Family	Rhodobacteraceae	32	0.01626992
Family	Phyllobacteriaceae	42	0.02135427
Family	Erythrobacteraceae	123	0.0625375
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Family	Idiomarinaceae	5	0.002542175
Family	Acetobacteraceae	68	0.03457358
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Family	Puniceicoccaceae	1	0.000508435
Family	Dehalobacteriaceae	1	0.000508435
Family	Polyangiaceae	1	0.000508435
Family	Hydrogenophilaceae	1	0.000508435
Family	Contubernalisaceae	1	0.000508435

Genus	Unclassified	6083	309.281
Genus	Pontibacter	8461	4.301.868
Genus	Oxalobacter	3657	1.859.347
Genus	Streptococcus	1399	0.7113005
Genus	Devosia	7810	3.970.877
Genus	Variovorax	3251	1.652.922
Genus	Pedobacter	11108	5.647.695
Genus	Pseudomonas	54228	2.757.141
Genus	Anaerofilum	639	0.3248899
Genus	Janthinobacterium	32743	1.664.769
Genus	Sphingomonas	7503	3.814.787
Genus	Campylobacter	6343	3.225.003
Genus	Methylotenera	2819	1.433.278
Genus	Kaistobacter	2789	1.418.025
Genus	Methylibium	3429	1.743.423
Genus	Helicobacter	5996	3.048.576
Genus	Agrobacterium	2322	1.180.586
Genus	Novosphingobium	1161	0.5902929
Genus	Kocuria	398	0.2023571
Genus	Comamonas	30	0.01525305
Genus	Microvirus	2495	1.268.545
Genus	Oscillospira	996	0.5064012
Genus	Mannheimia	3568	1.814.096
Genus	Paucibacter	222	0.1128726
Genus	Zoogloea	847	0.4306444
Genus	Blautia	2567	1.305.153
Genus	Escherichia	151	0.07677367
Genus	Rhodospirillum	568	0.288791
Genus	Akkermansia	49	0.02491331
Genus	Caulobacter	1260	0.640628
Genus	Phenylobacterium	1891	0.9614505
Genus	Propionibacterium	277	0.1408365
Genus	Desulfovibrio	825	0.4194588
Genus	Methylobacterium	416	0.2115089
Genus	Adhaeribacter	1186	0.6030039
Genus	Azospirillum	224	0.1138894
Genus	Butyrivibrio	2	0.00101687
Genus	Flavisolibacter	322	0.163716
Genus	Erwinia	174	0.08846768
Genus	Rhodocyclus	71	0.03609888
Genus	Ramlibacter	719	0.3655647
Genus	Asticcacaulis	96	0.04880976
Genus	Mucispirillum	205	0.1042292
Genus	Mycoplana	1133	0.5760568
Genus	Acholeplasma	32	0.01626992
Genus	Staphylococcus	1292	0.656898
Genus	Gemmatimonas	256	0.1301593
Genus	Alkaliphilus	540	0.2745549
Genus	Rhodoplanes	20	0.0101687
Genus	Arthrobacter	289	0.1469377

Genus	Dysgonomonas	197	0.1001617
Genus	Pseudoxanthomonas	305	0.1550727
Genus	Rubrivivax	448	0.2277789
Genus	Microbulbifer	17	0.008643394
Genus	Faecalibacterium	129	0.06558811
Genus	Paenibacillus	16	0.008134959
Genus	Parabacteroides	747	0.3798009
Genus	Xanthomonas	429	0.2181186
Genus	Slackia	79	0.04016636
Genus	Ruminococcus	569	0.2892995
Genus	Dietzia	43	0.0218627
Genus	Corynebacterium	824	0.4189504
Genus	Mesoplasma	10	0.005084349
Genus	Polynucleobacter	129	0.06558811
Genus	Herbaspirillum	620	0.3152297
Genus	Lachnospira	78	0.03965792
Genus	Bifidobacterium	12	0.006101219
Genus	Geobacillus	54	0.02745549
Genus	Cohnella	232	0.1179569
Genus	Johnsonella	466	0.2369307
Genus	Halomonas	176	0.08948455
Genus	Shinella	77	0.03914949
Genus	Luteimonas	167	0.08490863
Genus	Oribacterium	41	0.02084583
Genus	Rhodococcus	374	0.1901547
Genus	Aquicella	99	0.05033506
Genus	Sphingobacterium	7	0.003559045
Genus	Tetragenococcus	3	0.001525305
Genus	Klebsiella	118	0.05999532
Genus	Clostridium	723	0.3675984
Genus	Marinomonas	18	0.009151828
Genus	Mycoplasma	63	0.0320314
Genus	Bradyrhizobium	66	0.03355671
Genus	Rickettsia	14	0.007118089
Genus	Pelagicoccus	142	0.07219776
Genus	Lactobacillus	127	0.06457124
Genus	Burkholderia	230	0.11694
Genus	Caldithrix	8	0.00406748
Genus	Calothrix	280	0.1423618
Genus	Flavobacterium	142	0.07219776
Genus	Aeromicrobium	17	0.008643394
Genus	Coprococcus	211	0.1072798
Genus	Achromobacter	121	0.06152063
Genus	Enhydrobacter	150	0.07626524
Genus	Natronincola	17	0.008643394
Genus	Allobaculum	177	0.08999299
Genus	Polaromonas	27	0.01372774
Genus	Hymenobacter	70	0.03559044
Genus	Holdemania	112	0.05694471
Genus	Odoribacter	121	0.06152063

Genus	Cupriavidus	107	0.05440254
Genus	Hyphomonas	4	0.00203374
Genus	Bartonella	17	0.008643394
Genus	Brevundimonas	12	0.006101219
Genus	Agromyces	3	0.001525305
Genus	Roseburia	75	0.03813262
Genus	Rhodobacter	11	0.005592784
Genus	Mesorhizobium	9	0.004575914
Genus	Erythrobacter	10	0.005084349
Genus	Salinisphaera	45	0.02287957
Genus	Anaerobiospirillum	10	0.005084349
Genus	Vogesella	8	0.00406748
Genus	Gramella	2	0.00101687
Genus	Pseudidiomarina	5	0.002542175
Genus	Gluconobacter	11	0.005592784
Genus	Rhizobium	34	0.01728679
Genus	Phyllobacterium	33	0.01677835
Genus	Kushneria	3	0.001525305
Genus	Olivibacter	83	0.0422001
Genus	Demequina	1	0.000508435
Genus	Thalassospira	3	0.001525305
Genus	Dechloromonas	42	0.02135427
Genus	Ralstonia	12	0.006101219
Genus	Candidatus Glomeribacter	4	0.00203374
Genus	Sarcina	86	0.0437254
Genus	Arthrospira	149	0.0757568
Genus	Candidatus Phytoplasma	3	0.001525305
Genus	Marinobacter	44	0.02237114
Genus	Bacillus	15	0.007626524
Genus	Kribbella	1	0.000508435
Genus	Gallibacterium	9	0.004575914
Genus	Aurantimonas	19	0.009660264
Genus	Luteibacter	2	0.00101687
Genus	Xanthobacter	278	0.1413449
Genus	Mycobacterium	39	0.01982896
Genus	Geobacter	48	0.02440488
Genus	Delftia	12	0.006101219
Genus	Serratia	8	0.00406748
Genus	Acidimicrobium	4	0.00203374
Genus	Skermanella	1	0.000508435
Genus	Desulfuromusa	5	0.002542175
Genus	Acetobacterium	176	0.08948455
Genus	Lutibacterium	113	0.05745315
Genus	Thiomonas	15	0.007626524
Genus	Acidovorax	8	0.00406748
Genus	Sphingobium	14	0.007118089
Genus	Curvibacter	14	0.007118089
Genus	Catonella	28	0.01423618
Genus	Peptococcus	4	0.00203374
Genus	Flammeovirga	4	0.00203374

Genus	Dyadobacter	17	0.008643394
Genus	Chitinophaga	4	0.00203374
Genus	Leptolyngbya	1	0.000508435
Genus	Psychrobacter	5	0.002542175
Genus	Amaricoccus	8	0.00406748
Genus	Peptoniphilus	7	0.003559045
Genus	Megasphaera	1	0.000508435
Genus	Thiohalorhabdus	2	0.00101687
Genus	Pseudochrobactrum	37	0.01881209
Genus	Shewanella	9	0.004575914
Genus	Pasteurella	21	0.01067713
Genus	Chromobacterium	5	0.002542175
Genus	Nesterenkonia	1	0.000508435
Genus	Desulfonauticus	9	0.004575914
Genus	Methylobacillus	29	0.01474461
Genus	Thermodesulfovibrio	6	0.00305061
Genus	Emticicia	15	0.007626524
Genus	Legionella	9	0.004575914
Genus	Desulfotomaculum	10	0.005084349
Genus	Sporosarcina	4	0.00203374
Genus	Gallionella	4	0.00203374
Genus	Butyricimonas	6	0.00305061
Genus	Azohydromonas	3	0.001525305
Genus	Desulfobacter	3	0.001525305
Genus	Kutzneria	14	0.007118089
Genus	Azomonas	11	0.005592784
Genus	Bilophila	5	0.002542175
Genus	Azorhizobium	2	0.00101687
Genus	Roseospira	20	0.0101687
Genus	Bacteroides	6	0.00305061
Genus	Aquabacterium	23	0.011694
Genus	Enterobacter	2	0.00101687
Genus	Thiocapsa	4	0.00203374
Genus	Desulfomonile	10	0.005084349
Genus	Labrys	7	0.003559045
Genus	Thiorhodospira	5	0.002542175
Genus	Saccharopolyspora	3	0.001525305
Genus	Nitrosococcus	2	0.00101687
Genus	Deefgea	4	0.00203374
Genus	Ochrobactrum	8	0.00406748
Genus	Thioalkalimicrobium	4	0.00203374
Genus	Pseudoalteromonas	4	0.00203374
Genus	Caldilinea	8	0.00406748
Genus	Hydrogenophilus	5	0.002542175
Genus	Salinivibrio	1	0.000508435
Genus	Turicibacter	4	0.00203374
Genus	Methyloversatilis	16	0.008134959
Genus	Micromonospora	6	0.00305061
Genus	Cycloclasticus	2	0.00101687
Genus	Hydrogenophaga	18	0.009151828

Genus	Candidatus Liberibacter	4	0.00203374
Genus	Streptomyces	2	0.00101687
Genus	Coprobacillus	19	0.009660264
Genus	Thiothrix	4	0.00203374
Genus	Tolumonas	4	0.00203374
Genus	Rheinheimera	1	0.000508435
Genus	Rhodothermus	14	0.007118089
Genus	Ferrimicrobium	1	0.000508435
Genus	Virgibacillus	10	0.005084349
Genus	Alkalibacterium	8	0.00406748
Genus	Gemella	4	0.00203374
Genus	Anaerobranca	2	0.00101687
Genus	Kaistia	3	0.001525305
Genus	Rhodoferax	1	0.000508435
Genus	Acidiphilium	6	0.00305061
Genus	Glycomyces	3	0.001525305
Genus	Aquitalea	8	0.00406748
Genus	Leptothrix	14	0.007118089
Genus	Caldanaerobacter	1	0.000508435
Genus	Flexispira	4	0.00203374
Genus	Brachybacterium	2	0.00101687
Genus	Eggerthella	1	0.000508435
Genus	Bdellovibrio	3	0.001525305
Genus	Trichodesmium	1	0.000508435
Genus	Parapedobacter	10	0.005084349
Genus	Hydrocarboniphaga	6	0.00305061
Genus	Microbacterium	3	0.001525305
Genus	Prevotella	3	0.001525305
Genus	Moraxella	4	0.00203374
Genus	Psychromonas	1	0.000508435
Genus	Viridibacillus	1	0.000508435
Genus	Sedimentibacter	15	0.007626524
Genus	Runella	2	0.00101687
Genus	Desulfonatronum	6	0.00305061
Genus	Marinospirillum	3	0.001525305
Genus	Porphyromonas	14	0.007118089
Genus	Deinococcus	2	0.00101687
Genus	Lautropia	6	0.00305061
Genus	Martelella	5	0.002542175
Genus	Euzebya	9	0.004575914
Genus	Arthronema	2	0.00101687
Genus	Marivita	1	0.000508435
Genus	Ehrlichia	11	0.005592784
Genus	Eubacterium	1	0.000508435
Genus	Nisaea	1	0.000508435
Genus	Arcicella	1	0.000508435
Genus	Planococcus	1	0.000508435
Genus	Halanaerobium	1	0.000508435
Genus	Rickettsiella	3	0.001525305
Genus	Hyphomicrobium	9	0.004575914

Genus	Saccharospirillum	2	0.00101687
Genus	Paracoccus	3	0.001525305
Genus	Lentibacillus	3	0.001525305
Genus	Heliorestis	3	0.001525305
Genus	Candidatus Amoebophilus	7	0.003559045
Genus	Maricaulis	1	0.000508435
Genus	Macrococcus	3	0.001525305
Genus	Oerskovia	1	0.000508435
Genus	Chroococcus	1	0.000508435
Genus	Blastochloris	1	0.000508435
Genus	Kineococcus	1	0.000508435
Genus	Lysobacter	1	0.000508435
Genus	Trabulsiella	1	0.000508435
Genus	Thioalkalivibrio	2	0.00101687
Genus	Limnohabitans	9	0.004575914
Genus	Gillisia	2	0.00101687
Genus	Caldicellulosiruptor	1	0.000508435
Genus	Methylophaga	6	0.00305061
Genus	Arcanobacterium	2	0.00101687
Genus	Stenotrophomonas	8	0.00406748
Genus	Limnobacter	6	0.00305061
Genus	Nannocystis	2	0.00101687
Genus	Planifilum	1	0.000508435
Genus	Pediococcus	3	0.001525305
Genus	Adlercreutzia	2	0.00101687
Genus	Kitasatospora	2	0.00101687
Genus	Fervidobacterium	2	0.00101687
Genus	Jeotgalicoccus	3	0.001525305
Genus	Thermoanaerobacter	2	0.00101687
Genus	Borrelia	1	0.000508435
Genus	Candidatus Endobugula	1	0.000508435
Genus	Halorhodospira	1	0.000508435
Genus	Polaribacter	1	0.000508435
Genus	Erysipelothrix	11	0.005592784
Genus	Cystobacter	1	0.000508435
Genus	Alishewanella	8	0.00406748
Genus	Litoricola	3	0.001525305
Genus	Cellulomonas	1	0.000508435
Genus	Nitrincola	1	0.000508435
Genus	Lewinella	4	0.00203374
Genus	Acinetobacter	2	0.00101687
Genus	Leucothrix	2	0.00101687
Genus	Sutterella	1	0.000508435
Genus	Chryseobacterium	6	0.00305061
Genus	Candidatus Scalindua	1	0.000508435
Genus	Candidatus Blochmannia	2	0.00101687
Genus	Azoarcus	1	0.000508435
Genus	Ectothiorhodospira	4	0.00203374
Genus	Prosthecobacter	3	0.001525305
Genus	Candidatus Tammella	1	0.000508435

Genus	Sphingopyxis	3	0.001525305
Genus	Collimonas	1	0.000508435
Genus	Actinomyces	3	0.001525305
Genus	Amphritea	2	0.00101687
Genus	Anoxybacillus	4	0.00203374
Genus	Alkalibacillus	1	0.000508435
Genus	Thermogemmatispora	2	0.00101687
Genus	Thermodesulfatator	2	0.00101687
Genus	Nitrobacter	1	0.000508435
Genus	Myroides	1	0.000508435
Genus	Microcoleus	3	0.001525305
Genus	Tsukamurella	1	0.000508435
Genus	Marinitoga	1	0.000508435
Genus	Thermicanus	4	0.00203374
Genus	Roseomonas	1	0.000508435
Genus	Ancylobacter	2	0.00101687
Genus	Brenneria	1	0.000508435
Genus	Acidisoma	1	0.000508435
Genus	Actinokineospora	1	0.000508435
Genus	Steroidobacter	2	0.00101687
Genus	Leuconostoc	7	0.003559045
Genus	Leucobacter	1	0.000508435
Genus	Kineosporia	2	0.00101687
Genus	Haliscomenobacter	1	0.000508435
Genus	Desulfobulbus	1	0.000508435
Genus	Luteolibacter	3	0.001525305
Genus	Coraliomargarita	1	0.000508435
Genus	Streptacidiphilus	1	0.000508435
Genus	Neisseria	1	0.000508435
Genus	Denitratisoma	2	0.00101687
Genus	Pelotomaculum	2	0.00101687
Genus	Dehalobacterium	1	0.000508435
Genus	Chondromyces	1	0.000508435
Genus	Caloramator	2	0.00101687
Genus	Thiobacillus	1	0.000508435
Genus	Desulfosporosinus	1	0.000508435
Genus	Candidatus Contubernalis	1	0.000508435
Genus	Cellvibrio	1	0.000508435
Genus	Actinopolymorpha	2	0.00101687
Genus	Acidocella	1	0.000508435
Genus	Neorickettsia	2	0.00101687
Genus	Uliginosibacterium	1	0.000508435
Genus	Tepidanaerobacter	1	0.000508435
Genus	Oleomonas	1	0.000508435
Genus	Nocardioides	1	0.000508435
Genus	Deferribacter	2	0.00101687
Genus	Rhodovibrio	1	0.000508435
Genus	Rubellimicrobium	1	0.000508435
Genus	Candidatus Rhodoluna	1	0.000508435
Genus	Desulfovermiculus	1	0.000508435

Genus	Arenimonas	1	0.000508435
Genus	Fructobacillus	1	0.000508435
Genus	Arcobacter	1	0.000508435
Genus	Actinocatenispora	1	0.000508435
Genus	Azovibrio	1	0.000508435
Species	Unclassified	116044	5.900.082
Species	Pontibacter niistensis	174	0.08846768
Species	Oxalobacter vibrioformis	3656	1.858.838
Species	Variovorax boronicumulans	331	0.168292
Species	Pseudomonas corrugata	2633	1.338.709
Species	Sphingomonas oligophenolica	1405	0.7143511
Species	Pedobacter panaciterrae	9382	4.770.136
Species	Helicobacter mastomyrinus	206	0.1047376
Species	Kocuria rhizophila	340	0.1728679
Species	Variovorax paradoxus	2836	1.441.921
Species	Helicobacter mesocricetorum	960	0.4880975
Species	Pseudomonas brassicacearum	24243	1.232.599
Species	Microvirus Enterobacteria phage PhiX1	2495	1.268.545
Species	Mannheimia caviae	3568	1.814.096
Species	Zoogloea resiniphila	638	0.3243815
Species	Devosia hwasunensis	61	0.03101453
Species	Akkermansia muciniphila	49	0.02491331
Species	Janthinobacterium lividum	1219	0.6197822
Species	Caulobacter henricii	1183	0.6014785
Species	Janthinobacterium agaricidamnosum	1944	0.9883975
Species	Propionibacterium acnes	232	0.1179569
Species	Pseudomonas lini	812	0.4128492
Species	Methylobacterium jeotgali	197	0.1001617
Species	Blautia hansenii	368	0.1871041
Species	Azospirillum rugosum	222	0.1128726
Species	Butyrivibrio proteoclasticus	2	0.00101687
Species	Rhodocyclus purpureus	71	0.03609888
Species	Ramlibacter tataouinensis	212	0.1077882
Species	Asticcacaulis biprosthecium	82	0.04169166
Species	Pseudomonas plecoglossicida	478	0.2430319
Species	Pseudomonas mediterranea	193	0.09812795
Species	Mucispirillum schaedleri	205	0.1042292
Species	Helicobacter ganmani	1474	0.7494331
Species	Gemmatimonas aurantiaca	161	0.08185802
Species	Alkaliphilus crotonatoxidans	400	0.203374
Species	Caulobacter tundrae	41	0.02084583
Species	Arthrobacter psychrochitiniphilus	156	0.07931585
Species	Dysgonomonas wimpennyi	197	0.1001617
Species	Pseudomonas taiwanensis	209	0.1062629
Species	Pseudomonas azotoformans	464	0.2359138
Species	Sphingomonas dokdonensis	95	0.04830132
Species	Desulfovibrio butyratiphilus	232	0.1179569
Species	Methylobacterium marchantiae	9	0.004575914
Species	Methylotenera mobilis	99	0.05033506
Species	Pseudomonas agarici	231	0.1174485

Species	Pseudomonas clemancea	35	0.01779522
Species	Streptococcus pseudopneumoniae	137	0.06965559
Species	Streptococcus vestibularis	287	0.1459208
Species	Parabacteroides goldsteinii	661	0.3360755
Species	Pseudomonas entomophila	3597	182.884
Species	Xanthomonas oryzae	413	0.2099836
Species	Pseudomonas lundensis	374	0.1901547
Species	Erwinia mallotivora	152	0.07728211
Species	Ruminococcus gnavus	288	0.1464293
Species	Helicobacter apodemus	225	0.1143979
Species	Dietzia alimentaria	43	0.0218627
Species	Mesoplasma entomophilum	10	0.005084349
Species	Herbaspirillum chlorophenolicum	613	0.3116706
Species	Lachnospira pectinoschiza	78	0.03965792
Species	Methylotenera versatilis	143	0.07270619
Species	Geobacillus vulcani	42	0.02135427
Species	Cohnella soli	232	0.1179569
Species	Oscillospira eae	175	0.08897611
Species	Johnsonella ignava	465	0.2364222
Species	Kaistobacter terrae	182	0.09253516
Species	Halomonas fontilapidosi	162	0.08236646
Species	Luteimonas marina	150	0.07626524
Species	Oribacterium sinus	41	0.02084583
Species	Rhodococcus kroppenstedtii	118	0.05999532
Species	Sphingobacterium shayense	3	0.001525305
Species	Tetragenococcus doogicus	3	0.001525305
Species	Blautia coccoides	242	0.1230413
Species	Klebsiella granulomatis	9	0.004575914
Species	Devosia yakushimensis	167	0.08490863
Species	Mycoplasma insons	62	0.03152297
Species	Pseudomonas benzenivorans	52	0.02643862
Species	Lactobacillus gigeriorum	2	0.00101687
Species	Sphingomonas sanxanigenens	250	0.1271087
Species	Burkholderia seminalis	62	0.03152297
Species	Pseudomonas mosselii	69	0.03508201
Species	Streptococcus bovis	7	0.003559045
Species	Pedobacter kwangyangensis	247	0.1255834
Species	Staphylococcus pasteuri	324	0.1647329
Species	Pseudomonas teessidea	255	0.1296509
Species	Pseudomonas fuscovaginae	129	0.06558811
Species	Corynebacterium tuberculostearicum	101	0.05135193
Species	Burkholderia phenoliruptrix	39	0.01982896
Species	Pontibacter xinjiangensis	23	0.011694
Species	Pseudomonas parafulva	35	0.01779522
Species	Calothrix parietina	278	0.1413449
Species	Sphingomonas hunanensis	48	0.02440488
Species	Aeromicrobium alkaliterrae	12	0.006101219
Species	Staphylococcus auricularis	114	0.05796158
Species	Pseudomonas viridiflava	106	0.0538941
Species	Achromobacter insolitus	97	0.04931819

Species	Pseudomonas borealis	123	0.0625375
Species	Streptococcus thermophilus	438	0.2226945
Species	Corynebacterium stationis	168	0.08541707
Species	Corynebacterium doosanense	52	0.02643862
Species	Blautia wexlerae	77	0.03914949
Species	Luteimonas terricola	11	0.005592784
Species	Pseudoxanthomonas sacheonensis	50	0.02542175
Species	Sphingomonas elodea	23	0.011694
Species	Devosia chinhatensis	158	0.08033272
Species	Devosia geojensis	129	0.06558811
Species	Cupriavidus metallidurans	106	0.0538941
Species	Bartonella rochalimae	15	0.007626524
Species	Flavisolibacter ginsengisoli	15	0.007626524
Species	Brevundimonas staleyi	7	0.003559045
Species	Ruminococcus flavefaciens	79	0.04016636
Species	Novosphingobium aromaticivorans	2	0.00101687
Species	Roseburia faecis	75	0.03813262
Species	Pseudomonas vancouverensis	4	0.00203374
Species	Kocuria gwangalliensis	39	0.01982896
Species	Propionibacterium microaerophilum	16	0.008134959
Species	Burkholderia ubonensis	7	0.003559045
Species	Pseudomonas moraviensis	11	0.005592784
Species	Enhydrobacter aerosaccus	35	0.01779522
Species	Lactobacillus intestinalis	5	0.002542175
Species	Mesorhizobium camelthorni	9	0.004575914
Species	Erythrobacter aquimaris	10	0.005084349
Species	Corynebacterium casei	14	0.007118089
Species	Anaerobiospirillum succiniciproducens	10	0.005084349
Species	Agrobacterium larrymoorei	63	0.0320314
Species	Sphingomonas japonica	20	0.0101687
Species	Flavobacterium aquatile	15	0.007626524
Species	Vogesella perlucida	8	0.00406748
Species	Parabacteroides johnsonii	42	0.02135427
Species	Gluconobacter krungthepensis	9	0.004575914
Species	Rhizobium alamii	33	0.01677835
Species	Phenylobacterium mobile	34	0.01728679
Species	Thalassospira xianhensis	1	0.000508435
Species	Pseudomonas kilonensis	2	0.00101687
Species	Dechloromonas hortensis	10	0.005084349
Species	Novosphingobium indicum	2	0.00101687
Species	Aeromicrobium ponti	4	0.00203374
Species	Ralstonia insidiosa	12	0.006101219
Species	Brevundimonas olei	5	0.002542175
Species	Sphingomonas melonis	566	0.2877742
Species	Sarcina maxima	86	0.0437254
Species	Pseudomonas fluorescens	117	0.05948689
Species	Pseudomonas proteolytica	5	0.002542175
Species	Hymenobacter daecheongensis	12	0.006101219
Species	Candidatus Phytoplasma prunorum	1	0.000508435
Species	Marinomonas basaltis	3	0.001525305

Species	Sphingomonas panni	91	0.04626758
Species	Kribbella ginsengisoli	1	0.000508435
Species	Hymenobacter xinjiangensis	30	0.01525305
Species	Gallibacterium melopsittaci	9	0.004575914
Species	Aurantimonas litoralis	19	0.009660264
Species	Lactobacillus johnsonii	45	0.02287957
Species	Streptococcus tigurinus	18	0.009151828
Species	Oscillospira guilliermondii	23	0.011694
Species	Luteibacter anthropi	2	0.00101687
Species	Streptococcus fryi	32	0.01626992
Species	Xanthobacter viscosus	230	0.11694
Species	Sphingomonas insulae	90	0.04575915
Species	Novosphingobium stygium	22	0.01118557
Species	Klebsiella variicola	98	0.04982662
Species	Corynebacterium kroppenstedtii	14	0.007118089
Species	Mycobacterium frederiksbergense	31	0.01576148
Species	Streptococcus milleri	2	0.00101687
Species	Delftia lacustris	12	0.006101219
Species	Serratia entomophila	8	0.00406748
Species	Staphylococcus epidermidis	18	0.009151828
Species	Sphingobacterium bambusae	4	0.00203374
Species	Odoribacter denticanis	4	0.00203374
Species	Skermanella aerolata	1	0.000508435
Species	Desulfuromusa succinoxidans	3	0.001525305
Species	Staphylococcus hominis	72	0.03660731
Species	Sphingomonas soli	4	0.00203374
Species	Phyllobacterium catacumbae	26	0.01321931
Species	Thiomonas thermosulfata	11	0.005592784
Species	Agrobacterium albertimagni	22	0.01118557
Species	Acidovorax anthurii	5	0.002542175
Species	Arthrospira fusiformis	63	0.0320314
Species	Devosia limi	26	0.01321931
Species	Flavobacterium terrigena	3	0.001525305
Species	Pseudomonas mucidolens	9	0.004575914
Species	Sphingobium faniae	2	0.00101687
Species	Curvibacter gracilis	14	0.007118089
Species	Catonella morbi	28	0.01423618
Species	Peptococcus niger	4	0.00203374
Species	Clostridium caenicola	3	0.001525305
Species	Flammeovirga pacifica	4	0.00203374
Species	Desulfovibrio piger	33	0.01677835
Species	Chitinophaga soli	4	0.00203374
Species	Leptolyngbya laminosa	1	0.000508435
Species	Amaricoccus macauensis	1	0.000508435
Species	Peptoniphilus coxii	5	0.002542175
Species	Megasphaera hominis	1	0.000508435
Species	Thiohalorhabdus denitrificans	2	0.00101687
Species	Pseudomonas koreensis	17	0.008643394
Species	Pseudochrobactrum saccharolyticum	35	0.01779522
Species	Pasteurella pneumotropica	20	0.0101687

Species	Chromobacterium haemolyticum	3	0.001525305
Species	Nesterenkonia lacusekhoensis	1	0.000508435
Species	Herbaspirillum magnetovibrio	1	0.000508435
Species	Pontibacter korlensis	2	0.00101687
Species	Desulfonauticus autotrophicus	9	0.004575914
Species	Sphingomonas ginsenosidimutans	4	0.00203374
Species	Pseudomonas cinnamophila	10	0.005084349
Species	Helicobacter suncus	33	0.01677835
Species	Agromyces salentinus	1	0.000508435
Species	Methylobacillus glycogenes	29	0.01474461
Species	Thermodesulfovibrio thiophilus	6	0.00305061
Species	Methylobacterium goesingense	14	0.007118089
Species	Emticicia oligotrophica	15	0.007626524
Species	Olivibacter soli	2	0.00101687
Species	Desulfotomaculum indicum	10	0.005084349
Species	Sporosarcina pasteurii	4	0.00203374
Species	Gallionella ferruginea	4	0.00203374
Species	Herbaspirillum aquaticum	1	0.000508435
Species	Pseudomonas tremae	8	0.00406748
Species	Pseudomonas syncyanea	5	0.002542175
Species	Butyricimonas synergistica	6	0.00305061
Species	Geobacter toluenoxydans	2	0.00101687
Species	Azohydromonas australica	3	0.001525305
Species	Caulobacter crescentus	20	0.0101687
Species	Clostridium taeniosporum	2	0.00101687
Species	Escherichia albertii	21	0.01067713
Species	Lactobacillus antri	11	0.005592784
Species	Desulfomonile tiedjei	10	0.005084349
Species	Lactobacillus reuteri	18	0.009151828
Species	Saccharopolyspora shandongensis	2	0.00101687
Species	Nitrosococcus watsoni	2	0.00101687
Species	Rhodobacter gluconicum	2	0.00101687
Species	Variovorax ginsengisoli	1	0.000508435
Species	Ochrobactrum pseudogrignonense	6	0.00305061
Species	Sphingomonas mali	10	0.005084349
Species	Thioalkalimicrobium sibiricum	4	0.00203374
Species	Caldilinea tarbellica	8	0.00406748
Species	Helicobacter rodentium	7	0.003559045
Species	Clostridium thermosuccinogenes	28	0.01423618
Species	Lactobacillus taiwanensis	5	0.002542175
Species	Salinivibrio budaii	1	0.000508435
Species	Clostridium alkalicellulosi	15	0.007626524
Species	Sphingomonas suberifaciens	1	0.000508435
Species	Turicibacter sanguinis	3	0.001525305
Species	Escherichia coli	22	0.01118557
Species	Clostridium fallax	1	0.000508435
Species	Pseudomonas collierea	18	0.009151828
Species	Kushneria indalinina	1	0.000508435
Species	Phenylobacterium lituiforme	7	0.003559045
Species	Halomonas sediminis	1	0.000508435

Species	Methyloversatilis universalis	16	0.008134959
Species	Pseudomonas coronafaciens	12	0.006101219
Species	Hydrogenophilus denitrificans	3	0.001525305
Species	Cycloclasticus oligotrophus	2	0.00101687
Species	Rhodococcus corynebacterioides	5	0.002542175
Species	Amaricoccus kaplicensis	6	0.00305061
Species	Desulfovibrio fairfieldensis	3	0.001525305
Species	Hydrogenophaga defluvii	15	0.007626524
Species	Achromobacter arsenitoxydans	6	0.00305061
Species	Coprobacillus cateniformis	18	0.009151828
Species	Agrobacterium tumefaciens	2	0.00101687
Species	Pseudomonas umsongensis	5	0.002542175
Species	Arthrobacter rhombi	5	0.002542175
Species	Bifidobacterium bombi	3	0.001525305
Species	Tolumonas auensis	4	0.00203374
Species	Corynebacterium ammoniagenes	8	0.00406748
Species	Rheinheimera soli	1	0.000508435
Species	Rhodothermus clarus	14	0.007118089
Species	Ferrimicrobium acidiphilum	1	0.000508435
Species	Sphingomonas kwangyangensis	4	0.00203374
Species	Virgibacillus salexigens	10	0.005084349
Species	Xanthobacter polyaromaticivorans	36	0.01830366
Species	Lactobacillus vaginalis	5	0.002542175
Species	Gemella cunicula	4	0.00203374
Species	Pseudomonas frederiksbergensis	1	0.000508435
Species	Clostridium cadaveris	5	0.002542175
Species	Anaerobranca zavarzinii	2	0.00101687
Species	Pseudomonas metavorans	4	0.00203374
Species	Kaistia granuli	1	0.000508435
Species	Rhodoferax ferrireducens	1	0.000508435
Species	Acidiphilium acidophilum	5	0.002542175
Species	Aquitalea denitrificans	8	0.00406748
Species	Erwinia papayae	10	0.005084349
Species	Alkaliphilus peptidifermentans	11	0.005592784
Species	Leptothrix discophora	11	0.005592784
Species	Caldanaerobacter hydrothermalis	1	0.000508435
Species	Eggerthella sinensis	1	0.000508435
Species	Bdellovibrio exovorus	3	0.001525305
Species	Trichodesmium havanum	1	0.000508435
Species	Helicobacter rappini	9	0.004575914
Species	Bacteroides denticanum	1	0.000508435
Species	Parapedobacter koreensis	10	0.005084349
Species	Herbaspirillum seropedicae	1	0.000508435
Species	Prevotella dentasini	3	0.001525305
Species	Burkholderia brasilensis	6	0.00305061
Species	Asticcacaulis benevestitus	4	0.00203374
Species	Propionibacterium avidum	7	0.003559045
Species	Moraxella caviae	4	0.00203374
Species	Aquicella lusitana	1	0.000508435
Species	Viridibacillus neidei	1	0.000508435

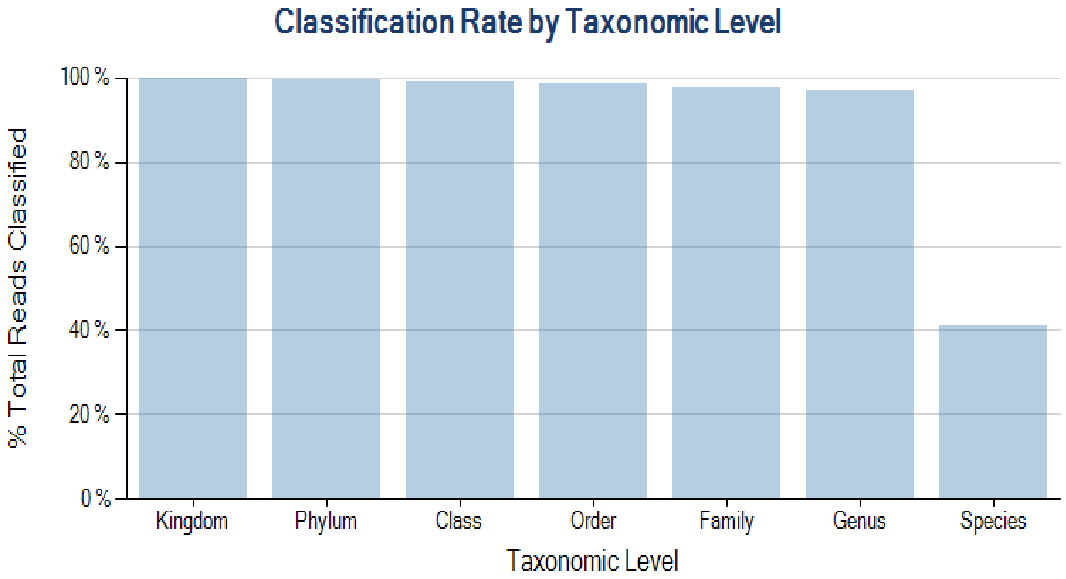
Species	Sedimentibacter hydroxybenzoicus	15	0.007626524
Species	Arthrobacter creatinolyticus	1	0.000508435
Species	Acidovorax wohlfahrtii	2	0.00101687
Species	Pseudomonas oryzihabitans	1	0.000508435
Species	Runella limosa	2	0.00101687
Species	Dechloromonas agitata	2	0.00101687
Species	Desulfonatronum thiosulfatophilum	6	0.00305061
Species	Thiomonas perometabolis	3	0.001525305
Species	Agrobacterium viscosum	16	0.008134959
Species	Acidovorax temperans	1	0.000508435
Species	Ochrobactrum thiophenivorans	1	0.000508435
Species	Flavobacterium algicola	3	0.001525305
Species	Porphyromonas canis	14	0.007118089
Species	Dechloromonas aromatica	10	0.005084349
Species	Lautropia mirabilis	6	0.00305061
Species	Euzebya tangerina	9	0.004575914
Species	Arthronema africanum	2	0.00101687
Species	Propionibacterium humerusii	9	0.004575914
Species	Streptococcus infantis	1	0.000508435
Species	Ehrlichia ovina	11	0.005592784
Species	Pseudomonas mendocina	3	0.001525305
Species	Clostridium aestuarii	5	0.002542175
Species	Nisaea nitritireducens	1	0.000508435
Species	Marinobacter arcticus	2	0.00101687
Species	Planococcus maritimus	1	0.000508435
Species	Halanaerobium alcaliphilum	1	0.000508435
Species	Hyphomicrobium aestuarii	7	0.003559045
Species	Agromyces terreus	1	0.000508435
Species	Microbulbifer okinawensis	1	0.000508435
Species	Lentibacillus salinarum	2	0.00101687
Species	Geobacillus gargensis	2	0.00101687
Species	Candidatus Amoebophilus asiaticus	7	0.003559045
Species	Maricaulis indicus	1	0.000508435
Species	Oerskovia ginkgo	1	0.000508435
Species	Chroococcus minutus	1	0.000508435
Species	Blastochloris gulmargensis	1	0.000508435
Species	Arthrobacter halodurans	2	0.00101687
Species	Kineococcus gynurae	1	0.000508435
Species	Pseudomonas lutea	8	0.00406748
Species	Trabulsiella odontotermitis	1	0.000508435
Species	Thioalkalivibrio jannaschii	2	0.00101687
Species	Staphylococcus aureus	6	0.00305061
Species	Limnohabitans parvus	8	0.00406748
Species	Deefgea rivuli	3	0.001525305
Species	Rhodobacter ovatus	3	0.001525305
Species	Kaistia soli	2	0.00101687
Species	Sphingomonas desiccabilis	1	0.000508435
Species	Erwinia psidii	1	0.000508435
Species	Novosphingobium acidiphilum	8	0.00406748
Species	Pseudomonas mandelii	2	0.00101687

Species	Arcanobacterium bernardiae	1	0.000508435
Species	Pedobacter agri	1	0.000508435
Species	Aquicella siphonis	5	0.002542175
Species	Limnobacter litoralis	6	0.00305061
Species	Shinella yambaruensis	4	0.00203374
Species	Sphingomonas abaci	1	0.000508435
Species	Devosia terrae	2	0.00101687
Species	Planifilum fimeticola	1	0.000508435
Species	Pediococcus cellicola	1	0.000508435
Species	Adlercreutzia equolifaciens	2	0.00101687
Species	Clostridium proteolyticus	1	0.000508435
Species	Desulfovibrio psychrotolerans	4	0.00203374
Species	Campylobacter faecalis	2	0.00101687
Species	Slackia piriformis	1	0.000508435
Species	Novosphingobium hassiacum	1	0.000508435
Species	Asticcacaulis taihuensis	1	0.000508435
Species	Lactobacillus gasseri	4	0.00203374
Species	Fervidobacterium pennivorans	2	0.00101687
Species	Jeotgalicoccus halotolerans	1	0.000508435
Species	Mycoplasma haemominutum	1	0.000508435
Species	Pseudomonas pavonaceae	6	0.00305061
Species	Peptoniphilus methioninivorax	1	0.000508435
Species	Clostridium bovipellis	2	0.00101687
Species	Halorhodospira halochloris	1	0.000508435
Species	Polaribacter butkevichii	1	0.000508435
Species	Pseudochrobactrum kiredjianiae	2	0.00101687
Species	Erysipelothrix muris	9	0.004575914
Species	Pseudomonas poae	2	0.00101687
Species	Nitrincola lacisaponensis	1	0.000508435
Species	Pseudomonas putida	7	0.003559045
Species	Lactobacillus siliginis	4	0.00203374
Species	Lewinella marina	1	0.000508435
Species	Lactobacillus senmaizukei	2	0.00101687
Species	Rhodococcus percolatus	1	0.000508435
Species	Acinetobacter xiamenensis	1	0.000508435
Species	Microbulbifer epialgicus	2	0.00101687
Species	Marinomonas brasiliensis	2	0.00101687
Species	Leucothrix mucor	2	0.00101687
Species	Sphingobium abikonense	4	0.00203374
Species	Pseudomonas amygdali	5	0.002542175
Species	Herbaspirillum frisingense	1	0.000508435
Species	Sphingobium olei	2	0.00101687
Species	Corynebacterium flavescens	1	0.000508435
Species	Variovorax soli	2	0.00101687
Species	Sutterella sanguinus	1	0.000508435
Species	Chryseobacterium taichungense	6	0.00305061
Species	Desulfovibrio carbinolicus	1	0.000508435
Species	Jeotgalicoccus coquinae	2	0.00101687
Species	Candidatus Scalindua brodae	1	0.000508435
Species	Mycobacterium pinnipedii	1	0.000508435

Species	Hydrogenophaga taeniospiralis	1	0.000508435
Species	Enterobacter amnigenus	1	0.000508435
Species	Ectothiorhodospira imhoffii	1	0.000508435
Species	Prosthecobacter fluviatilis	1	0.000508435
Species	Ectothiorhodospira haloalkaliphila	3	0.001525305
Species	Candidatus Tammella caduceiae	1	0.000508435
Species	Sphingopyxis granuli	3	0.001525305
Species	Collimonas pratensis	1	0.000508435
Species	Pseudomonas gessardii	2	0.00101687
Species	Helicobacter cynogastricus	2	0.00101687
Species	Pseudomonas brenneri	1	0.000508435
Species	Actinomyces naturae	3	0.001525305
Species	Amphritea atlantica	2	0.00101687
Species	Anoxybacillus amylolyticus	4	0.00203374
Species	Herbaspirillum rubrisubalbicans	1	0.000508435
Species	Alkalibacterium subtropicum	2	0.00101687
Species	Alkalibacillus salilacus	1	0.000508435
Species	Sphingomonas fennica	2	0.00101687
Species	Thermodesulfatator atlanticus	2	0.00101687
Species	Nitrobacter alkalicus	1	0.000508435
Species	Microcoleus antarcticus	3	0.001525305
Species	Bradyrhizobium jicamae	1	0.000508435
Species	Rhodococcus equi	1	0.000508435
Species	Rickettsia monacensis	1	0.000508435
Species	Helicobacter brantae	2	0.00101687
Species	Acidisoma tundrae	1	0.000508435
Species	Actinokineospora inagensis	1	0.000508435
Species	Steroidobacter denitrificans	2	0.00101687
Species	Variovorax dokdonensis	2	0.00101687
Species	Sphingomonas echinoides	1	0.000508435
Species	Leucobacter chironomi	1	0.000508435
Species	Arthrobacter soli	1	0.000508435
Species	Bifidobacterium choerinum	1	0.000508435
Species	Gluconobacter morbifer	1	0.000508435
Species	Corynebacterium halotolerans	1	0.000508435
Species	Staphylococcus caprae	2	0.00101687
Species	Lewinella lutea	1	0.000508435
Species	Pediococcus argentinicus	2	0.00101687
Species	Desulfobulbus elongatus	1	0.000508435
Species	Devosia ginsengisoli	2	0.00101687
Species	Luteolibacter algae	2	0.00101687
Species	Coraliomargarita akajimensis	1	0.000508435
Species	Desulfovibrio litoralis	1	0.000508435
Species	Corynebacterium nuruki	2	0.00101687
Species	Novosphingobium mathurense	2	0.00101687
Species	Streptacidiphilus griseus	1	0.000508435
Species	Lactobacillus frumenti	1	0.000508435
Species	Corynebacterium argentoratense	1	0.000508435
Species	Staphylococcus fleurettii	1	0.000508435
Species	Acholeplasma cavigenitalium	1	0.000508435

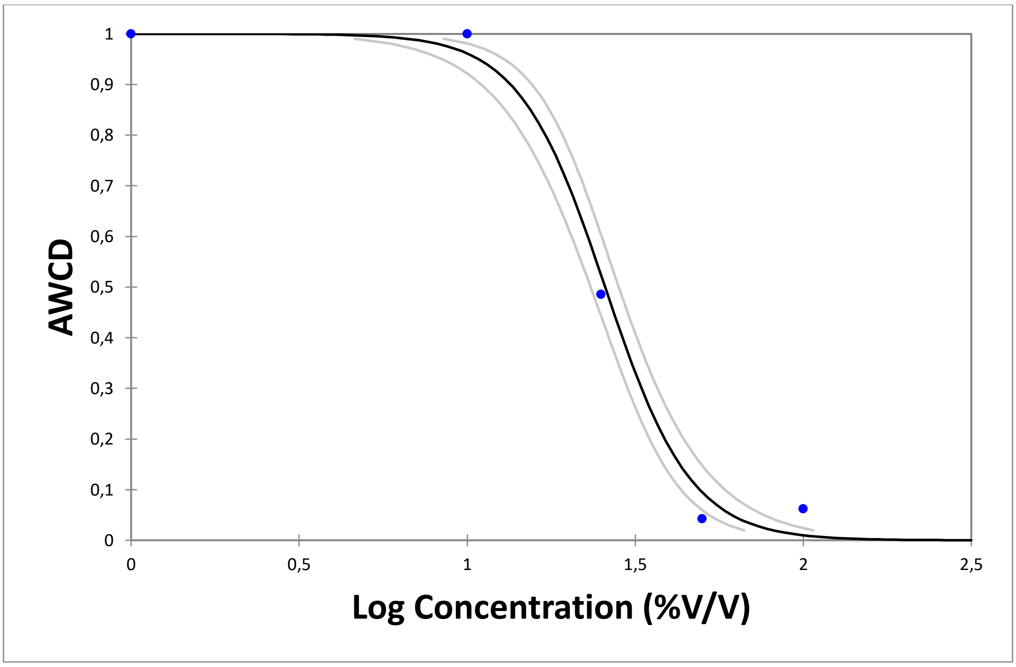
Species	Neisseria mucosa	1	0.000508435
Species	Roseospira visakhapatnamensis	1	0.000508435
Species	Denitratisoma oestradiolicum	2	0.00101687
Species	Luteimonas composti	1	0.000508435
Species	Chromobacterium piscinae	1	0.000508435
Species	Clostridium tepidiprofundi	2	0.00101687
Species	Pedobacter koreensis	1	0.000508435
Species	Hyphomonas oceanitis	1	0.000508435
Species	Pelotomaculum isophthalicicum	2	0.00101687
Species	Desulfovibrio aceae	1	0.000508435
Species	Chondromyces pediculatus	1	0.000508435
Species	Stenotrophomonas retroflexus	1	0.000508435
Species	Acinetobacter antiviralis	1	0.000508435
Species	Pseudomonas japonica	1	0.000508435
Species	Candidatus Contubernalis alkalaceticur	1	0.000508435
Species	Cellvibrio ostraviensis	1	0.000508435
Species	Halomonas almeriensis	1	0.000508435
Species	Halomonas shengliensis	1	0.000508435
Species	Xanthomonas albilineans	1	0.000508435
Species	Polaromonas naphthalenivorans	1	0.000508435
Species	Acidocella aluminiidurans	1	0.000508435
Species	Neorickettsia helminthoeca	2	0.00101687
Species	Pedobacter daejeonensis	1	0.000508435
Species	Halomonas sabkhae	1	0.000508435
Species	Uliginosibacterium gangwonense	1	0.000508435
Species	Corynebacterium ulceribovis	2	0.00101687
Species	Tepidanaerobacter syntrophicus	1	0.000508435
Species	Oleomonas sagaranensis	1	0.000508435
Species	Nocardioides islandensis	1	0.000508435
Species	Sphingomonas aquatilis	1	0.000508435
Species	Deferribacter autotrophicus	2	0.00101687
Species	Rhodovibrio sodomensis	1	0.000508435
Species	Clostridium histolyticum	1	0.000508435
Species	Helicobacter trogontum	1	0.000508435
Species	Streptomyces lazureus	1	0.000508435
Species	Rubellimicrobium aerolatum	1	0.000508435
Species	Lactobacillus intermedius	1	0.000508435
Species	Hydrogenophaga intermedia	1	0.000508435
Species	Desulfovermiculus halophilus	1	0.000508435
Species	Allobaculum stercoricanis	2	0.00101687
Species	Arenimonas malthae	1	0.000508435
Species	Fructobacillus pseudoficulneus	1	0.000508435
Species	Methylophaga alcalica	1	0.000508435
Species	Clostridium termitidis	1	0.000508435
Species	Arcobacter marinus	1	0.000508435
Species	Actinocatenispora silicis	1	0.000508435
Species	Corynebacterium acetoacidophilum	1	0.000508435
Species	Lentibacillus kapialis	1	0.000508435
Species	Staphylococcus chromogenes	1	0.000508435
Species	Candidatus Liberibacter solanacearum	1	0.000508435

Species	Azovibrio restrictus	1	0.000508435
Species	Streptococcus gallinaceus	1	0.000508435
Species	Brachybacterium squillarum	1	0.000508435
Species	Marinobacter santoriniensis	1	0.000508435
Species	Paenibacillus thailandensis	1	0.000508435



Taxonomic Level	Reads Classified to Taxonomic Level	% Total Reads Classified to Taxonomic Level
Kingdom	196,495	99.90 %
Phylum	195,528	99.41 %
Class	194,996	99.14 %
Order	193,976	98.62 %
Family	192,532	97.89 %
Genus	190,599	96.91 %
Species	80,638	41.00 %

S.I. 2. Percentage of microorganisms by taxonomic level of a sample collected from a natural soil (Zaragoza, NE Spain).



SI 3. The black curve shows the AWCD values of metabolized substrates in Biolog EcoPlates after exposure (24 h) of soil bacteria to *A. absinthium* L hydrolate as a function of logarithm of the concentration. G*rey lines* are the confidence limits (95%). Curve is the average value of three replicates. AWCD values are expressed as the percentage of the control.