BIODEGRADATION OF EMERGING CONTAMINANTS IN AGRICULTURAL SOILS AND THEIR IMPACT ON SOIL MICROBIAL COMMUNITIES

By

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ABSTRACT

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The incomplete elimination of pharmaceuticals and personal care products (PPCPs) during wastewater treatment has resulted in their dissemination in agricultural soils. Biodegradation is a potential removal mechanism; however, the microorganisms and pathways involved are generally unknown. The current work examined the biodegradation of carbamazepine (CBZ), diclofenac (DCF), triclocarban (TCC), and triclosan (TCS) in agricultural soils under aerobic and anaerobic conditions. Solid phase extraction and liquid chromatography tandem mass spectrometry were used for PPCP extraction and analysis. The soil microbial communities were investigated using 16S rRNA gene amplicon and shotgun sequencing.

The first study examined CBZ biodegradation at three concentrations (50, 500, 5000 ng/g), under aerobic and anaerobic conditions, in two soils, using 16S rRNA gene amplicon sequencing and an approach to predict metagenomes (phylogenetic investigation of communities by reconstruction of unobserved states, PICRUSt). The most significant CBZ biodegradation occurred under aerobic conditions. PICRUSt revealed that one soil contained a greater abundance of xenobiotic degrading genes. Several phylotypes were enriched following CBZ degradation, including unclassified *Sphingomonadaceae*, *Xanthomonadaceae* and *Rhodobacteraceae*, as well as *Sphingomonas*, *Aquicella* and *Microvirga*. These phylotypes are considered putative CBZ degraders as they appear to be benefiting from CBZ biodegradation.

The second study focused on DCF, CBZ and TCC biodegradation in four soils at concentrations typically detected in soils and biosolids (50 ng g^{-1}) using 16S rRNA gene

amplicon sequencing and PICRUSt. Rapid DCF removal (<7 days) was observed under aerobic conditions, with limited biodegradation under other conditions. CBZ and TCC degradation was slow (half-lives of 128-241 days and 165-190 days for CBZ and TCC). Phylotypes in the *Proteobacteria, Gemmatimonadales* and *Actinobacteria* were more abundant during DCF biodegradation. For CBZ, those in the *Bacteroidetes, Actinobacteria, Proteobacteria* and *Verrucomicrobia* were enriched during biodegradation. *Actinobacteria* and *Proteobacteria* were also enriched during TCC biodegradation. Such differences could indicate these microorganisms are associated with biodegradation. The impact on KEGG (Kyoto Encyclopedia of Genes and Genomes) metabolism pathways was also examined. Four pathways were positively impacted during DCF biodegradation. CBZ had a limited impact on the metabolic pathways. TCC removal was linked to genes associated with the degradation of simple and complex substrates.

The third study examined CBZ, TCC and TCS biodegradation using shotgun sequencing and MG-RAST analysis. CBZ and TCC biodegradation was again slow, and TCS biodegradation was rapid. For each chemical, between three and ten phylotypes were enriched during biodegradation. The genera of previously reported CBZ, TCC or TCS degrading isolates were present; *Rhodococcus* (CBZ), *Streptomyces* (CBZ), *Pseudomonas* (CBZ, TCC, TCS), *Sphingomonas* (TCC, TCS), *Methylobacillus* (TCS) and *Stenotrophomonas* (TCS). From the analysis of xenobiotic degrading pathways, five KEGG pathways were the most dominant.

This research indicates a number of phylotypes are likely involved in PPCP biodegradation in agricultural soils. Also, the work suggests that the phylotypes impacted are affected by the experimental conditions (e.g. PPCP concentration, soil type, incubation time). From the PPCPs examined, CBZ and TCC are highly recalcitrant and will likely remain in agricultural soils for extended periods of time.

To my dear wife Lita for her love and support.
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KEY TO ABBREVIATIONS

CBZ	Carbamazepine
DCF	Diclofenac
KBS LTER	Kellogg Biological Station Long-Term Ecological Research
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MG-RAST	Metagenomics Rapid Annotation using Subsystem Technology
OTU	Operational Taxonomic Unit
PAH	Polycyclic aromatic hydrocarbon
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
PPCPs	Pharmaceuticals and personal care products
QuEChERS	Quick, easy, cheap, effective, rugged & safe
RTSF	Research Technology Support Facility
SPE	Solid phase extraction
STAMP	Statistical Analysis of Taxonomic and Functional Profiles
TCC	Triclocarban
TCS	Triclosan
TEAP	Terminal electron-accepting processes
WWTP	Wastewater treatment plant

Chapter 1:

Introduction

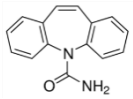
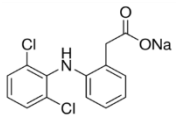
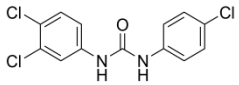
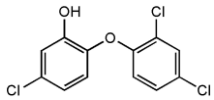
This chapter presents some background on the occurrence of pharmaceuticals and personal care products (PPCPs) in biosolids and agricultural soils. It also discusses high throughput sequencing and the bioinformatics tools for processing and analyzing the sequencing data. The chapter concludes with a summary and the objectives of the three research chapters (Chapters 2-4).

1.1. Pharmaceuticals and Personal Care Products in Biosolids

The release of PPCPs into the environment has become a major problem because of the known or suspected adverse effects of these bioactive compounds. Irrigation with wastewater and biosolids application constitute the primary routes of entry of PPCPs into the environment (Daughton and Ternes, 1999; Wu et al., 2010b). During the wastewater treatment process, these compounds undergo partial removal (Cha and Cupples, 2009; Clara et al., 2005; Miao and Metcalfe, 2003) leading to their presence in the wastewater effluents and biosolids (Wu et al., 2010b). Land application of biosolids provides a beneficial way to dispose of sludge while simultaneously supplying nutrients to the receiving soils, as well as improving their physical, chemical, and biological properties (Lu et al., 2012). Despite the benefits of such a practice, the growing use of biosolids as agricultural amendments has raised concern, in part, because of the presence of various pollutants, often called contaminants of emerging concern. Among contaminants commonly found in biosolids are the anti-seizure drug carbamazepine (5H-dibenzo [b, f] azepine-5-carboxamide) (CBZ), the non-steroidal anti-inflammatory drug diclofenac (2-(2, 6-dichloranilino) phenylacetic acid) (DCF) and two antibmicrobial agents, triclocarban (3-(4-

chlorophenyl)-1-(3, 4-dichlorophenyl) urea (TCC) and triclosan (2, 4, 4′-trichloro-2′-hydroxydiphenyl ether) (TCS) (Table 1.1).

Table 1.1 Selected chemical and physical properties of the pharmaceuticals and personal care products used in this study (Chefetz et al., 2008; Loftsson et al., 2005; Vazquez-Roig et al., 2010).

Compound name	Biological activity	Structure	Molecular weight (g mol^{-1})	LogKow	pKa	Water solubility (mg/L)
CBZ	Anticonvulsant		236.27	2.45	13.9	125
DCF	Anti-inflammatory		296.16	4.51	4.15	360
TCC	Antimicrobial agent		315.6	4.9	12.7	< 0.05
TCS	Antimicrobial agent		289.5	4.8	7.9	≤1

In a nationwide survey (2001) by the US Environmental Protection Agency (EPA), CBZ, TCC, and TCS were detected in biosolids from 94 US wastewater treatment plants located in 32 states plus the District of Columbia, with TCC and TCS representing 65% of the aggregate mass of contaminants (McClellan and Halden, 2010). Generally, the concentrations of CBZ, DCF, TCC, and TCS in biosolids vary from low ng g^{-1} to mg kg^{-1}. According to several reports, CBZ biosolids concentrations varied from 5 ng g^{-1} to up to 258 ng g^{-1} (Ding et al., 2011; Gottschall et al., 2012; Morais et al., 2013; Radjenovic et al., 2009a; Sabourin et al., 2012; Spongberg and Witter, 2008). DCF has been detected in biosolids at concentrations varying from ≤ 10 to

627 ng g^{-1} (Albero et al., 2014; Morais et al., 2013; Radjenovic et al., 2009b). Several research groups reported concentrations of 90 to 51,000 ng g^{-1} for TCC and TCS (Cha and Cupples, 2009; Chu and Metcalfe, 2007; Sabourin et al., 2012; Wu et al., 2010a). The presence of CBZ, DCF, TCC, and TCS in biosolids has the potential to cause a number of environmental problems, including CBZ teratogenicity (Matalon et al., 2002), DCF toxicity to birds (Hussain et al., 2008), and TCC and TCS endocrine disruption (Ahn et al., 2008; Bevans et al., 1996; Harries et al., 1996). Other environmental concerns include the translocation and bioaccumulation of these xenobiotics in plants. Several studies indicated the bioaccumulation of CBZ, DCF, TCC, and TCS in plants leaves/stems and roots (Carter et al., 2014; Dodgen et al., 2013; Holling et al., 2012; Shenker et al., 2011; Wu et al., 2010b; Wu et al., 2015). Considering the use of biosolids as amendment is a practice that will continue in the future, studying the fate of these problematic xenobiotics in the environment is warranted.

1.2. Pharmaceuticals and Personal Care Products in Soils

Several studies reported the occurrence of CBZ, DCF, TCC, and TCS in soils irrigated with wastewater or amended with biosolids (Cha and Cupples, 2009; Dalkmann et al., 2014; Duran-Alvarez et al., 2009; Higgins et al., 2011; Vazquez-Roig et al., 2010; Wu et al., 2010a). For instance, concentrations ranging from 0.16 to 200 ng g^{-1} have been reported for these PPCPs in soils (Cha and Cupples, 2009; Chen et al., 2011; Ding et al., 2011; Gibson et al., 2010; Vazquez-Roig et al., 2010; Walker et al., 2012; Wu et al., 2010a). Several research groups have described CBZ, DCF, TCC, and TCS as being persistent in soils (Dalkmann et al., 2014; Grossberger et al., 2014; Thelusmond et al., 2018; Thelusmond et al., 2016). The foregoing makes it necessary to better understand the processes that control the fate of these chemicals in agricultural soils in order to characterize the risks associated with their occurrence thereof.

Processes whereby these xenobiotics can be removed from the environment have been indicated, with biodegradation being an important process (Jones et al., 2001). However, most of the studies on the biodegradation of these PPCPs have focused on their biological transformation during wastewater treatment. Recently, researchers have started to investigate the sorption and degradation of these PPCPs in soils, and the findings reveal some variability regarding the PPCPs degradation rates. These trends are likely a result of soil physico-chemical and microbial characteristics along with the physicochemical properties each PPCP (Grossberger et al., 2014; Lin and Gan, 2011; Xu et al., 2009). Other factors that impact the fate of these PPCPs in soils include the availability of oxygen, prior exposure of the soils to PPCPs, and the initial concentration of the PPCPs under consideration (Grossberger et al., 2014; Lin and Gan, 2011; Xu et al., 2009). Although these studies have increased our knowledge regarding the biodegradation of the PPCPs in soils, there still exist some knowledge gaps that need to be addressed. For example, to date, the majority of CBZ, DCF, TCC, and TCS biodegradation studies in soils focus on aerobic conditions. Only a few studies have addressed the biodegradation of these chemicals under anaerobic conditions. Though soil aerobiosis tends to prevail in most agricultural soils (Tiedje et al., 1984), soil anaerobiosis exists in various pedologic settings such as wetlands, paddy soils, organic soils, poorly drained and heavy textured soils, soils with high water table, soils amended with manure, and soils fertilized with ammonia (Inglett et al., 2006). Anaerobic conditions do not solely prevail in the foregoing soil settings; in fact, even the so-called aerobic soils can periodically become anaerobic once oxygen consumption or supply changes as a result of some disturbances (e.g. flooding, soil compaction, and large application of manure (Tiedje et al., 1984). To address this knowledge gap, the

biodegradation of CBZ, DCF, TCC, and TCS was examined in the current study under aerobic (all PPCPs) and anaerobic (CBZ, DCF) conditions in agricultural soils.

1.3. High Throughput Sequencing to Investigate Microbial Communities

Soil microorganisms perform various essential biogeochemical processes in many ecosystems (Coleman et al., 1983; Gougoulias et al., 2014; Jacoby et al., 2017; Zhou et al., 2015) including the degradation of xenobiotics (Bao et al., 2017; Lovley, 2003; Malik et al., 2008). Standard culture techniques consisting of enriching and isolating pure cultures via growth media has been the method of choice for characterizing microbial community until the 1980s (Chakraborty et al., 2014). However, the standard culture techniques present some limitations that only an infinitesimal fraction of the microbial population (<1%) can be cultured using the standards methods (Torsvik and Ovreas, 2002). To address this limitation, molecular-based methods have emerged as alternative methods to characterize microbial communities without the need of cultivation. Molecular-based techniques offer the benefits of extracting useful metabolic and functional information on microbial communities without disrupting or disturbing the communities (Chakraborty et al., 2014). Among these methods, next generation sequencing or high throughput sequencing is becoming the method of choice for characterizing microbial communities (Laudadio et al., 2018). Unlike Sanger sequencing, developed in the late 1970s' (Sanger et al., 1977), next generation sequencing produces significantly more data in a shorter time span (Chakraborty et al., 2014). Other limitations of the Sanger sequencing technology include the need for a cloned-based library (Medini et al., 2008). Next generation sequencing typically takes two forms: 16S ribosomal RNA gene amplicon sequencing or shotgun sequencing (Laudadio et al., 2018; Techtmann and Hazen, 2016). 16S rRNA is an important nucleic acid based technique for studying microbial community composition and diversity in complex

habitats (Hill et al., 2000). 16S rRNA gene sequencing is advantageous due to its ubiquity in all forms of life, its highly conserved regions (Woese et al., 1990), its relative large size (~1.5 kb), and its secondary structures (Hill et al., 2000). The possibility of accessing various reference sequence and taxonomies databases of interest (greengenes, SILVa, and the Ribosomal Database Project) is another benefit in using 16S rRNA marker genes (Kuczynski et al., 2011). When the 16S rRNA approach is employed in microbial profiling, DNA is extracted from the environmental samples; then the amplification of the 16S rRNA gene is conducted using primers barcoded with oligonucleotides (Zhou et al., 2015). Despite the usefulness of the16S rRNA as a profiling phylogentic marker gene, it is unable to directly identify metabolic or other functional capabilities of microorganisms of interest in a given community. To curb these limitations, recently, a bioinformatics software package was designed to predict metagenome functional content from the 16S rRNA marker gene using a database of reference genomes. This computational approach, known as the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States or PICRUSt (Langille et al., 2013) uses evolutionary modeling to predict the functional composition of a metagenome.

An alternative method to 16S rRNA amplicon sequencing is whole genome shotgun sequencing (Sharpton, 2014) where random primers are used for the sequencing of overlapping regions of the genome. Shotgun sequencing allows a more extensive examination of a microbial community and the identification of all genes present (Chen and Pachter, 2005).

In any study involving high throughput sequencing, DNA extraction from the environmental samples is typically followed by the acquisition of sequence data from the extracted DNA. To this end, various sequencing technologies including the widely used Illumina/Solexa Genome Analyzer sequencing platform are employed (Mardis, 2008). After the acquisition of DNA

sequences, the next step is to analyze the acquired sequences. For targeted amplicon sequencing (e.g. 16 S rRNA), bioinformatics tools such as Mothur (Schloss et al., 2009) are typically used for comparing and analyzing microbial community (Kuczynski et al., 2011). Mothur enables the trimming, screening, and alignment of the sequences as well as the calculation of distances. It also assigns sequences to operational taxonomic unit (OTUs), in other words it assigns DNA sequences to microbial species (Kuczynski et al., 2011). As for functional annotation of shotgun sequences, one convenient way to analyze the data is to upload files onto the online platform Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST) (Meyer et al., 2008). Both 16S rRNA gene amplicon sequencing (Illumina MiSeq) coupled with Mothur and PICRUSt (Chapters 2 and 3), as well as shotgun sequencing (Illumina HiSeq) with MG-RAST (Chapter 4) were used in the research described here.

1.4. Dissertation Outline and Objectives

The overall objective of this dissertation was to examine the impact of CBZ, DCF, TCC, and TCS on the phylogenetic and functional characteristics of soil microbial communities. The thesis contains the chapters described below:

Chapter 2:

This chapter (published reference: Thelusmond, J.R., Strathmann, T.J., Cupples, A.M. 2016. The identification of carbamazepine biodegrading phylotypes and phylotypes sensitive to carbamazepine exposure in two soil microbial communities. Science of the Total Environment. 571, 1241-1252) describes experiments that examined the degradation of CBZ (50, 500 and 5000 ng g^{-1}) in two agricultural soils at three concentrations under aerobic and anaerobic conditions. In addition, the soil microbial community and functional composition of the metagenomes were evaluated using 16S rRNA gene amplicon sequencing data (Illumina MiSeq), Mothur, PICRUSt,

and STAMP (statistical analysis of taxonomic and functional profiles) (Parks et al., 2014). STAMP provides statistical tests for analyzing taxonomic and functional profiles.

The objectives were:

To examine CBZ biodegradation in two agricultural soils under aerobic and anaerobic conditions at three CBZ concentrations.

To determine which phylotypes increased in abundance following CBZ biodegradation, and could therefore be putatively associated with CBZ degradation.

To investigate the effects of CBZ and soil conditions on the inferred PICRUSt metagenomes.

Chapter 3:

This chapter (published reference: Thelusmond, J.R., Kawka, E., Strathmann, T.J., Cupples, A.M. 2018. Diclofenac, carbamazepine and triclocarban biodegradation in agricultural soils and the microorganisms and metabolic pathways affected. Science of the Total Environment. 640, 1393-1410) describes the biodegradation of DCF, CBZ and TCC in four agricultural soils along with the phylotypes and metabolic pathways impacted using 16S rRNA gene amplicon sequencing and PICRUSt. The removal of DCF, CBZ, and TCC in agricultural soils was examined at concentrations more typical of those encountered in soils (50 ng g^{-1}). While CBZ and TCC biodegradation was only investigated under aerobic conditions, DCF biodegradation was investigated under four different electron accepting conditions (O_2, NO_3^- reducing, SO_4^{2-} reducing, and methanogenic).

The objectives were:

To determine the susceptibility of DCF, CBZ, and TCC to biodegradation in different agricultural soils under aerobic and anaerobic conditions at environmentally relevant concentrations.

To identify which microbial phylotypes enriched during DCF, CBZ, and TCC biodegradation and therefore may be associated with the biodegradation of these chemicals.

To determine which metabolic pathways are associated with DCF, CBZ, and TCC biodegradation in agricultural soils.

Chapter 4:

The work described in Chapter 4 (submitted as Thelusmond, J.R., Strathmann, T.J., Cupples, A.M. Carbamazepine, triclocarban and triclosan biodegradation and the phylotypes and functional genes associated with xenobiotic degradation in four agricultural soils) involved shotgun sequencing to determine the phylotypes and the functional genes and pathways associated with the biodegradation of CBZ, TCC, and TCS. In the two previous chapters, 16S rRNA gene amplicon sequencing was coupled with a computational approach (PICRUSt) to determine the predicted metagenomes in soils during PPCP biodegradation. The work described in Chapter 4 adopted a different approach (shotgun sequencing and MG-RAST analysis) to examine soils subjected to different cropping and management systems. These soils were obtained from the Main Cropping System Experiment at Kellogg Biological Station Long-Term Ecological Research (KBS LTER).

The objectives were:

To determine the existence of specific phylotypes associated with CBZ, TCC and TCS degradation in agricultural soils subjected to different cropping and agronomic management systems using shotgun sequencing.

To determine which genes are associated with CBZ, TCC and TCS biodegradation in soils using shotgun sequencing.

To compare the phylotypes and functional genes associated with the xenobiotic degrading pathways in the soils and examine if the cropping regimes impacted the functional abilities of the soil microbial communities. Chapter 5: This is the concluding chapter wherein the major findings and future work are presented.

REFERENCES

REFERENCES

Ahn, K.C., Zhao, B., Chen, J., Cherednichenko, G., Sanmarti, E., Denison, M.S., et al. 2008. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens. Environmental Health Perspectives. 116, 1203-1210.

Albero, B., Sanchez-Brunete, C., Miguel, E., Aznar, R., Tadeo, J.L. 2014. Determination of selected pharmaceutical compounds in biosolids by supported liquid extraction and gas chromatography-tandem mass spectrometry. Journal of Chromatography A. 1336, 52-58.

Bao, Y.J., Xu, Z., Li, Y., Yao, Z., Sun, J., Song, H. 2017. High-throughput metagenomic analysis of petroleum-contaminated soil microbiome reveals the versatility in xenobiotic aromatics metabolism. Journal of Environmental Sciences (China). 56, 25-35.

Bevans, H.E., Goodbred, S.L., Miesner, J.F., Watkins, S.A., Gross, T.S., Denslow, N.D., et al. 1996. Synthetic organic compounds and carp endocrinology and histology in Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, 1992 and 1995. U.S. Geological Survey, Water-Resources Investigations Report., 96-4266.

Carter, L.J., Harris, E., Williams, M., Ryan, J.J., Kookana, R.S., Boxall, A.B. 2014. Fate and uptake of pharmaceuticals in soil-plant systems. Journal of Agricultural and Food Chemistry. 62, 816-25.

Cha, J.M., Cupples, A.M. 2009. Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids. Water Research. 43, 2522-2530.

Chakraborty, A., DasGupta, C.K., Bhadury, P. 2014. Application of Molecular Techniques for the Assessment of Microbial Communities in Contaminated Sites. Microbial Biodegradation and Bioremediation, 85-113.

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

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

Chen, K., Pachter, L. 2005. Bioinformatics for whole-genome shotgun sequencing of microbial communities. PLoS Computational Biology. 1, 106-12.

Chu, S., Metcalfe, C.D. 2007. Simultaneous determination of triclocarban and triclosan in municipal biosolids by liquid chromatography tandem mass spectrometry. Journal of Chromatography A. 1164, 212-218.

Coleman, D.C., Reid, C.P.P., Cole, C.V. 1983. Biological Strategies of Nutrient Cycling in Soil Systems. Advances in Ecological Research. 13, 1-55.

Dalkmann, P., Siebe, C., Amelung, W., Schloter, M., Siemens, J. 2014. Does long-term irrigation with untreated wastewater accelerate the dissipation of pharmaceuticals in soil? Environmental Science & Technology. 48, 4963-4970.

Ding, Y.J., Zhang, W.H., Gu, C., Xagoraraki, I., Li, H. 2011. Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. Journal of Chromatography A. 1218, 10-16.

Dodgen, L.K., Li, J., Parker, D., Gan, J.J. 2013. Uptake and accumulation of four PPCP/EDCs in two leafy vegetables. Environmental Pollution. 182, 150-156.

Duran-Alvarez, J.C., Becerril-Bravo, E., Castro, V.S., Jimenez, B., Gibson, R. 2009. The analysis of a group of acidic pharmaceuticals, carbamazepine, and potential endocrine disrupting compounds in wastewater irrigated soils by gas chromatography-mass spectrometry. Talanta. 78, 1159-1166.

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

Gottschall, N., Topp, E., Metcalfe, C., Edwards, M., Payne, M., Kleywegt, S., et al. 2012. Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field. Chemosphere. 87, 194-203.

Gougoulias, C., Clark, J.M., Shaw, L.J. 2014. The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. Journal of the Science of Food and Agriculture. 94, 2362-2371.

Grossberger, A., Hadar, Y., Borch, T., Chefetz, B. 2014. Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. Environmental Pollution. 185, 168-177.

Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, P., Routledge, E.J., et al. 1996. A Survey of estrogenic activity in United Kingdom inland waters. Environmental Toxicology and Chemistry. 15, 1993–2002.

Higgins, C.P., Paesani, Z.J., Chalew, T.E.A., Halden, R.U., Hundal, L.S. 2011. Persistence of triclocarban and triclosan in soils after land application of biosolids and bioaccumulation in Eisenia Foetida. Environmental Toxicology and Chemistry. 30, 556-563.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., et al. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology. 15, 25-36.

Holling, C.S., Bailey, J.L., Heuvel, B.V., Kinney, C.A. 2012. Uptake of human pharmaceuticals and personal care products by cabbage (*Brassica campestris*) from fortified and biosolids-amended soils. Journal of Environmental Monitoring. 14, 3029-3036.

Hussain, I., Khan, M.Z., Khan, A., Javed, I., Saleemi, M.K. 2008. Toxicological effects of diclofenac in four avian species. Avian Pathology. 37, 315-321.

Inglett, P.W., Reddy, K.R., Corstanje, R. 2006. Anaerobic Soils. University of Florida, Gainesville, FL, USA.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., Kopriva, S. 2017. The Role of Soil Microorganisms in Plant Mineral Nutrition-Current Knowledge and Future Directions. Frontiers in Plant Science. 8.

Jones, O.A.H., Voulvoulis, N., Lester, J.N. 2001. Human pharmaceuticals in the aquatic environment - A review. Environmental Technology. 22, 1383-1394.

Kuczynski, J., Lauber, C.L., Walters, W.A., Parfrey, L.W., Clemente, J.C., Gevers, D., et al. 2011. Experimental and analytical tools for studying the human microbiome. Nature Reviews Genetics. 13, 47-58.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., et al. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology. 31, 814-821.

Laudadio, I., Fulci, V., Palone, F., Stronati, L., Cucchiara, S., Carissimi, C. 2018. Quantitative Assessment of Shotgun Metagenomics and 16S rDNA Amplicon Sequencing in the Study of Human Gut Microbiome. Omics-a Journal of Integrative Biology. 22, 248-254.

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

Loftsson, T., Össurardóttir, Í.B., Thorsteinsson, T., Duan, M., Másson, M. 2005. Cyclodextrin Solubilization of the Antibacterial Agents Triclosan and Triclocarban: Effect of Ionization and Polymers. Journal of Inclusion Phenomena and Macrocyclic Chemistry. 52, 109-117.

Lovley, D.R. 2003. Cleaning up with genomics: applying molecular biology to bioremediation. Nature Reviews Microbiology. 1, 35-44.

Lu, Q., He, Z.L., Stoffella, P.J. 2012. Land Application of Biosolids in the USA: A Review. Applied and Environmental Soil Science. 2012, 1-11.

Malik, S., Beer, M., Megharaj, M., Naidu, R. 2008. The use of molecular techniques to characterize the microbial communities in contaminated soil and water. Environment International. 34, 265-276.

Mardis, E.R. 2008. Next-generation DNA sequencing methods. Annual Review of Genomics and Human Genetics. 9, 387-402.

Matalon, S., Schechtman, S., Goldzweig, G., Ornoy, A. 2002. The teratogenic effect of carbamazepine: a meta-analysis of 1255 exposures. Reproductive Toxicology. 16, 9-17.

McClellan, K., Halden, R.U. 2010. Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. Water Research. 44, 658-68.

Medini, D., Serruto, D., Parkhill, J., Relman, D.A., Donati, C., Moxon, R., et al. 2008. Microbiology in the post-genomic era. Nature Reviews Microbiology. 6, 419-30.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., Kubal, M., et al. 2008. The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics. 9, 386.

Morais, S.A., Delerue-Matos, C., Gabarrell, X., Blanquez, P. 2013. Multimedia fate modeling and comparative impact on freshwater ecosystems of pharmaceuticals from biosolids-amended soils. Chemosphere. 93, 252-262.

Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G. 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 30, 3123-3124.

Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., Segata, N. 2017. Shotgun metagenomics, from sampling to analysis. Nature Biotechnology. 35, 833-844.

Radjenovic, J., Jelic, A., Petrovic, M., Barcelo, D. 2009a. Determination of pharmaceuticals in sewage sludge by pressurized liquid extraction (PLE) coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Analytical and Bioanalytical Chemistry. 393, 1685-1695.

Radjenovic, J., Petrovic, M., Barcelo, D. 2009b. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Research. 43, 831-841.

Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D.R., Topp, E. 2012. Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. Science of the Total Environment. 431, 233-236.

Sanger, F., Nicklen, S., Coulson, A.R. 1977. DNA Sequencing with Chain-Terminating Inhibitors. Proceedings of the National Academy of Sciences of the United States of America. 74, 5463-5467.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537-7541.

Sharpton, T.J. 2014. An introduction to the analysis of shotgun metagenomic data. Frontiers in Plant Science. 5, 209.

Shenker, M., Harush, D., Ben-Ari, J., Chefetz, B. 2011. Uptake of carbamazepine by cucumber plants--a case study related to irrigation with reclaimed wastewater. Chemosphere. 82, 905-910.

Spongberg, A.L., Witter, J.D. 2008. Pharmaceutical compounds in the wastewater process stream in Northwest Ohio. Science of the Total Environment. 397, 148-157.

Techtmann, S.M., Hazen, T.C. 2016. Metagenomic applications in environmental monitoring and bioremediation. Journal of Industrial Microbiology & Biotechnology. 43, 1345-1354.

Thelusmond, J.R., Kawka, E., Strathmann, T.J., Cupples, A.M. 2018. Diclofenac, carbamazepine and triclocarban biodegradation in agricultural soils and the microorganisms and metabolic pathways affected. Science of the Total Environment. 640, 1393-1410.

Thelusmond, J.R., Strathmann, T.J., Cupples, A.M. 2016. The identification of carbamazepine biodegrading phylotypes and phylotypes sensitive to carbamazepine exposure in two soil microbial communities. Science of the Total Environment. 571, 1241-1252.

Tiedje, J.M., Sexstone, A.J., Parkin, T.B., Revsbech, N.P., Shelton, D.R. 1984. Anaerobic Processes in Soil. Plant and Soil. 76, 197-212.

Torsvik, V., Ovreas, L. 2002. Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology. 5, 240-5.

Vazquez-Roig, P., Segarra, R., Blasco, C., Andreu, V., Pico, Y. 2010. Determination of pharmaceuticals in soils and sediments by pressurized liquid extraction and liquid chromatography tandem mass spectrometry. Journal of Chromatography A. 1217, 2471-2483.

Walker, C.W., Watson, J.E., Williams, C. 2012. Occurrence of carbamazepine in soils under different land uses receiving wastewater. Journal of Environmental Quality. 41, 1263-1267.

Woese, C.R., Kandler, O., Wheelis, M.L. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proceedings of the National Academy of Sciences of the United States of America. 87, 4576-9.

Wu, C., Spongberg, A.L., Witter, J.D., Fang, M., Ames, A., Czajkowski, K.P. 2010a. Detection of pharmaceuticals and personal care products in agricultural soils receiving biosolids application. CLEAN - Soil, Air, Water. 38, 230-237.

Wu, C., Spongberg, A.L., Witter, J.D., Fang, M., Czajkowski, K.P. 2010b. Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water. Environmental Science & Technology. 44, 6157-6161.

Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J. 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Science of the Total Environment. 536, 655-666.

Xu, J., Wu, L.S., Chang, A.C. 2009. Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere. 77, 1299-1305.

Zhou, J.Z., He, Z.L., Yang, Y.F., Deng, Y., Tringe, S.G., Alvarez-Cohen, L. 2015. High-Throughput Metagenomic Technologies for Complex Microbial Community Analysis: Open and Closed Formats. Mbio. 6

Chapter 2:

The Identification of Carbamazepine Biodegrading Phylotypes and Phylotypes Sensitive to Carbamazepine Exposure in Two Soil Microbial Communities

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2.1. Abstract

Carbamazepine (CBZ), an antiepileptic drug, has been introduced into agricultural soils via irrigation with treated wastewater and biosolids application. Such contamination is problematic because CBZ is persistent and the risks to ecosystems or human health are unknown. The current study examined CBZ biodegradation in two agricultural soils (soil 1 and 2) and the effects on the soil microbial communities during CBZ exposure. The experimental design involved three CBZ concentrations (50, 500, 5000 ng g^{-1}), under aerobic as well as anaerobic conditions. CBZ concentration was determined using solid phase extraction and LC-MS/MS. The effect of CBZ on the soil microbial community was investigated using high throughput sequencing and a computational approach to predict functional composition of the metagenomes (phylogenetic investigation of communities by reconstruction of unobserved states, PICRUSt). The most significant CBZ biodegradation occurred in soil 1 under aerobic conditions. In contrast, CBZ biodegradation was limited under anaerobic conditions in soil 1 and under both conditions in soil 2. For soil 1, several phylotypes were enriched following CBZ degradation compared to the controls, including unclassified *Sphingomonadaceae*, *Xanthomonadaceae* and

Rhodobacteraceae, as well as *Sphingomonas*, *Aquicella* and *Microvirga*. These phylotypes are considered putative CBZ degraders as they appear to be benefiting from CBZ biodegradation. PICRUSt revealed that soil 1 contained a greater abundance of xenobiotic degrading genes compared to soil 2, and thus, this analysis method offers a potential valuable approach for predicting CBZ attenuation in soils. PICRUSt analysis also implicated *Sphingomonadaceae* and *Xanthomonadaceae* in drug metabolism. Interestingly, numerous phylotypes decreased in abundance following CBZ exposure and these varied with soil type, concentration, duration of exposure, and the availability of oxygen. For three phylotypes (*Flavobacterium*, 3 *genus incertae sedis* and *unclassified Bacteroidetes*), the relative abundance was reduced in both soils, indicating a notable sensitivity to CBZ for these microorganisms.

2.2. Introduction

It is now widely recognized that pharmaceuticals and personal care products (PPCPs) are not fully eliminated during wastewater treatment and can be released into the environment via wastewater effluent and biosolids (Cha and Cupples, 2009; Chen et al., 2011; Clara et al., 2004; Gottschall et al., 2012; Kinney et al., 2006b; Lajeunesse et al., 2012; Miao et al., 2005; Miege et al., 2008; Ollers et al., 2001; Vieno and Sillanpaa, 2014; Williams and McLain, 2012; Wu et al., 2010). This is a cause for concern because these chemicals are being detected in surface water and groundwater (Gottschall et al., 2012; Kolpin et al., 2002; Lapworth et al., 2012; Nakada et al., 2008; Tixier et al., 2003; Tran et al., 2014). The reuse of wastewater for irrigation of agricultural areas can also increase the presence PPCPs in the environment (Dalkmann et al., 2014; Gibson et al., 2010; Grossberger et al., 2014; Kinney et al., 2006a). The long term ecotoxicity potential of individual and mixtures of PPCPs in the environment has not been determined (Rosi-Marshall and Royer, 2012). The continued acceptance of 1) biosolids for

agricultural land application and 2) wastewater effluent reuse in arid and semi-arid areas will depend on the ecological and human risks associated with the fate and effect of PCPPs in the environment.

From the many PPCPs released into the environment, carbamazepine (5*H*-dibenzo [*b*,*f*]azepine-5-carboxamide, CBZ), an antiepileptic drug, is perhaps one of the most problematic. CBZ has been detected in many wastewater treatment plant (WWTP) samples (Metcalfe et al., 2003; Miao and Metcalfe, 2003; Ternes, 1998; Tixier et al., 2003). It was found in all influent and effluent samples from 18 WWTPs across Canada (Metcalfe et al., 2003). Another study detected CBZ in 30 WWTP effluents in Germany (Ternes, 1998). CBZ has also been detected in biosolids from WWTPs (Gottschall et al., 2012; Kinney et al., 2006b; Miao et al., 2005). CBZ was observed in nine different biosolids products in seven states with average concentrations ranging from 8 to 390 µg Kg^{-1} (Kinney et al., 2006b). A study involving the analysis of 110 biosolids samples suggested the projected land application rate of CBZ is 550-680 Kg yr^{-1} (McClellan and Halden, 2010). The frequency of detection measured for CBZ in this study was 100% and the mean concentration was 163 µg kg^{-1}. Thus, the frequent detection of CBZ in WWTP effluent and biosolids raises concerns about the fate and risks associated with applying treated wastewater for irrigation and land applying biosolids during crop production.

Several researchers have documented the recalcitrant nature of CBZ in soil following irrigation with wastewater effluent. For example, CBZ was one of the four most commonly detected pharmaceuticals in soil irrigated with reclaimed wastewater (Kinney et al., 2006a). In another study, CBZ exhibited half-live values ranging from 147 to >200 days following irrigation with treated wastewater (Grossberger et al., 2014). Further, soils exposed to CBZ through irrigation with untreated wastewater exhibited half-lives of 355-1,624 days (Dalkmann

et al., 2014). CBZ has also been shown to be highly persistent in soils following the applications of biosolids. In outdoor mesocosms with municipal biosolids, CBZ exhibited an estimated half-life of 462-533 days (Walters et al., 2010). CBZ was found in dewatered municipal biosolids aggregates incorporated into soil, one year post application at a level of 30 ng g^{-1} (Gottschall et al., 2012). A recent manuscript reported that CBZ mineralization did not exceed 2% of the spiked rate through 120 days of aerobic incubation in different soils (Li et al., 2013). Others have also reported the recalcitrant nature of CBZ when present in soils (Maeng et al., 2011; Wu et al., 2010).

Surprisingly, given the common occurrence of CBZ, very little is known about the microorganisms able to degrade this compound. To our knowledge, only two reports have identified bacteria capable of CBZ degradation. *Rhodococcus rhodochrous* and *Aspergillus niger* were reported to remove 15% and 9% respectively when exposed to ~10 mg L^{-1} CBZ (Gauthier et al., 2010). Also, *Streptomyces* MIUG 4.89 degraded up to 14% CBZ with an initial concentration of 0.2 mg L^{-1} (Popa et al., 2014). Notably, no information exists as to which microorganisms might be responsible for CBZ degradation in soils. Further, no data is available linking specific bacteria to CBZ degradation at environmentally relevant concentrations.

The current research was designed to determine which phylotypes increased in abundance following CBZ biodegradation, and could therefore be putatively associated with CBZ degradation. Our hypothesis being that these microorganisms are obtaining a growth benefit from CBZ degradation, perhaps as a carbon, nitrogen or energy source. The phylotypes linked to CBZ biodegradation in two agricultural soils, at three CBZ concentrations (50 ng g^{-1}, 500 ng g^{-1} and 5000 ng g^{-1}), under aerobic and anaerobic conditions, were investigated. Further, PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) was used to

identify important xenobiotic degradative genes under each treatment. PICRUSt is a computational approach that uses evolutional modeling to predict the functional composition of a metagenome using 16S rRNA gene data and a database of reference genomes (Langille et al., 2013). To our knowledge, this is the first attempt to identify CBZ degraders in soil communities.

The current research also included a secondary objective, which involved identifying the phylotypes detrimentally affected by CBZ. Although it is widely accepted that PPCPs are released into the environment, their unintended effects on receiving environments remain largely unknown. Exposure to PPCPs may alter the microbial community composition, which may in turn influence ecosystem function. For example, the expression of gene categories related to N, P and C cycling were strongly affected by the presence of pharmaceutical products (Yergeau et al., 2012). To date, research on the effects of PPCPs in soil microbial communities has primarily focused on the effects of antibiotics (Schmitt et al., 2004; Thiele-Bruhn, 2003; Thiele-Bruhn and Beck, 2005). Limited work has addressed on the impact of CBZ on microbial communities. The common occurrence of CBZ in wastewater effluent and biosolids and the recalcitrant nature of CBZ in soils suggest CBZ will continue to be a chemical of concern to many ecosystems. To address this knowledge gap, the current work examined the impact of CBZ on two soil microbial communities under both aerobic and anaerobic conditions. This is the first study on the impact of this chemical on specific soil microorganisms.

2.3. Methods

2.3.1. Chemicals and Materials

Carbamazepine (100%) and carbamazepine-d_{10} (99.4%) were purchased from Sigma-Aldrich (Saint-Louis, MO, USA) and C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada), respectively. Acetonitrile (HPLC grade) and methanol (Optima™ LC/MS) were purchased from

Fisher Scientific. QuEChERS (quick, easy, cheap, effective, rugged & safe) extract pouches (6.0 g magnesium sulfate and 1.5 g sodium acetate) were obtained from Agilent Technologies (Wilmington, DE, USA). Cleanert SAX cartridges (1000 mg, 6 mL) and Oasis HLB cartridges (200 mg, 6cc) were purchased from GS-Tek (Newark, DE, USA) and Waters (Milford, MA, USA), respectively. Stock CBZ solutions (100 mg L^{-1}) were prepared in DI water and stored at 4 ^{o}C until use. Working CBZ solutions (0.5 mg L^{-1}, 5.0 mg L^{-1}, and 50 mg L^{-1}) were also prepared through dilution with an appropriate volume of the stock solution in DI water. The solution concentrations were chosen to ensure the same volume (500 µL) of CBZ solution was added to each microcosm. Agricultural soils were collected from close to the campus of Michigan State University (42º46′22″N, 84º21′25″W) and were stored in the dark at 4^{o}C until use. Select physical and chemical properties were determined by A& L Great Lakes Laboratories, Inc. (Fort Wayne, IN) (Supplementary Table 2.1).

2.3.2. Experimental Setup

Soil samples were stored at 4^{0}C until use and were sieved (2-mm diameter) to remove the coarse particles. Background CBZ in the two soils (soils 1 and 2) were below the detection limit (<0.39 ng g^{-1}). Sacrificial incubation experiments were performed to measure CBZ dissipation in the two soils and evaluate the effects of a range of initial CBZ concentrations (50 ng g^{-1}, 500 ng g^{-1}, and 5000 ng g^{-1}) on the soil microbial community under both aerobic and anaerobic conditions.

The aerobic microcosms were established in amber glass serum bottles (30 mL) with 5.0 g (wet weight) of soil spiked with 500 µL of each CBZ working stock solution to obtain 50 ng g-1, 500 ng g^{-1}, or 5000 ng g^{-1} of CBZ g^{-1} soil. After CBZ addition, the contents were vortexed to mix the target PPCP throughout the sample. Then, the moisture content of each soil was adjusted to

60% of maximum water holding capacity (63 and 78 mL g^{-1} dry soil for soil 1 and 2). The aerobic microcosms were set up in triplicates and were opened periodically to enable oxygen replenishment. The anaerobic microcosms were established in similar manner with slight modifications. Briefly, 5.0 g of soil (wet weight) spiked with 500 µL of each CBZ solution was vortexed before adding 10 mL DI water into each bottle. The water was added to imitate saturated conditions, typical after a heavy precipitation event. The headspace was purged with oxygen-free nitrogen for 10 minutes and the bottles were immediately sealed with septa and crimps. The anaerobic microcosms were set up in triplicates or duplicates and remained closed for the duration of the experiment. Live controls (no CBZ controls) for both aerobic and anaerobic microcosms were prepared in a similar manner with the exclusion of CBZ from the 500 µL DI water spiking solution.

To assess the role of microorganisms in the degradation of CBZ, abiotic controls were also included (for soil 1 only under aerobic conditions, as soil 2 illustrated no significant degradation). For this, 5.0 g of soil (wet weight) were placed in serum bottles capped with aluminum foil; the bottles and their contents were autoclaved at 121^{o}C for 45 minutes for three consecutive days after cooling. CBZ solutions were filtered (22 micron nylon syringe filter) and the DI water used to adjust the soil maximum water holding capacity was also autoclaved at 121^{o}C for 45 minutes.

All bottles were kept in the dark until they were sampled. CBZ was extracted (as described below) from the sacrificed microcosms and the abiotic controls on days 4 and 14. Before CBZ extraction, an aliquot of soil (0.35 g of wet soil) was taken from each sample and each live control for total DNA extraction (as described below).

Another set of experiments was performed to assess the long-term biodegradation potential of CBZ (at 50 ng g^{-1}) in soil 1. For this, CBZ concentrations were determined at days 3, 14, 33 and 50 for triplicates of live and abiotic controls under both aerobic and anaerobic conditions. The experimental setup was the same as that described above, except DNA was not extracted.

2.3.3. CBZ Extraction and Analytical Methods

Methods were developed to extract CBZ from soils using a modified QuEChERS approach based on previous work (Salvia et al., 2012). The CBZ internal standard (CBZ-d_{10}) was spiked (200 ng g^{-1}) to the soil matrix before extraction. The extraction procedure consisted of adding 10 mL of DI water plus 15 mL of acetonitrile to the soil samples. The mixture was vortexed for 2 min; then slowly and continuously, the QuEChERS buffer (6 g of magnesium sulfate and 1.5 g of sodium acetate) was added while vortexing at a low speed (Bragança et al. 2012). The tubes were manually shaken for 30 sec and vortexed at high speed for 30 sec. The tubes were subsequently placed on a shaker (Lab-Line, Lab-Line Instruments, Inc. Melrose, IL) at 750 rpm for 3 min before centrifugation at 3000 *g* (2 min). Then, 10 mL of the acetonitrile supernatant was placed in a 12-mL glass tube. Finally, the extract was evaporated to dryness through a gentle stream of nitrogen at 40 ^{o}C and reconstituted with 60 mL of methanol-water (3:97, v/v). The pH of the extract was adjusted to an average of 7.3. The above technique was used to extract CBZ from all the aerobic as well as the anaerobic microcosms. However, only acetonitrile was added to the anaerobic microcosms since water was already present (each sample already contained 10 mL DI water). To determine CBZ extraction efficiency, 5.0 g of soil (wet weight) was placed in 50 mL polypropylene tubes and samples were spiked with 500 µL of CBZ in methanol (5 mg L^{-1}) to obtain 500 ng g^{-1} of CBZ g^{-1} soil. Then, the above CBZ extraction method was followed.

2.3.4. Processing of Soil Extracts

The reconstituted soil extracts underwent a clean-up step prior to their analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). The solid phase extraction (SPE) technique presented in this work is a modification of the SPE reported by Salvia et al. (2012) and Vazquez-Roig et al. (2010) to purify soil extracts containing pharmaceuticals. First, a strong anion exchange cartridge (SAX) was placed in tandem with a polymeric cartridge (Oasis HLB). The SAX cartridge was used to minimize matrix interferences due to the presence of humic and fulvic acids in the soil extract (Vazquez-Roig et al., 2010). Next, both cartridges were preconditioned successively with methanol (5 mL) and deionized water (5 mL). Then the reconstituted soil extracts (60 mL) were loaded into the cartridges. The bottles containing the soil extracts were rinsed with 10 mL of DI water, which was then loaded into the cartridges. The cartridges were subsequently washed with 5 mL of DI water before being dried under vacuum for 30 min. The SAX cartridges were removed prior to eluting CBZ from the HLB cartridges. The elution solvent consisted of 2 x 5 mL of methanol. The flow rate for both the loading and elution steps was < 1 mL min^{-1}. The collected eluate was evaporated to dryness under a stream of nitrogen at 40^{o}C and reconstituted with 1 mL of methanol before transferring to 1.5 mL HPLC vials and storing at -15^{o}C until LC-MS/MS analysis.

2.3.5. LC-MS/MS Analysis

Mass spectrometry was performed using an LC-MS/MS system comprising a Shimadzu high performance liquid chromatograph (HPLC, Columbia, MD, USA) coupled with an API SCIEX 3200 triple quadrupole mass spectrometer (Foster City, CA, USA) equipped with TurboIonSpray ion source. CBZ and CBZ-d_{10} were ionized in positive mode with ion spray voltage and temperature set to 5000 V and 750 ^{o}C, respectively. The curtain gas pressure was set to 10 psi,

whereas the collision gas pressure and the ion source gas pressure were set to 9 psi and 30 psi, respectively. The MS/MS parameters were optimized for both CBZ and CBZ-d10 (Supplementary Table 2.2). The HPLC system utilized comprised of two LC-20AD pumps, an SIL-20A autosampler and a DGU-20A degasser with a CBM-20A controller. Gemini C18 column (50 x 2.00 mm, 5µm, Phenomenex) was used for the LC separation. The mobile phase consisted of 0.3% formic acid in MilliQ water (A) and acetonitrile (B) with a flow rate of 0.35 mL/min. A gradient involved 10% of eluent B for 0.5 min; then the eluent B underwent an increase to 100% between 0.5-2 min and was held constant between 2-3 min. Finally, a decrease from 100% to 10% was effected between 3-3.5 min. The injection volume per sample was 10 µL and the equilibration time between run was set to 2 min. The average recovery for CBZ in soil was 103.78 ± 1.42% for the tested concentration (500 ng g^{-1}).

2.3.6. DNA Extraction, MiSEQ Illumina Sequencing and PICRUSt

DNA was extracted using the Power Soil DNA extraction kit (MO BIO Laboratories, Inc. Carlsbad, CA) following the manufacturer's instructions. DNA was extracted from replicate microcosms on day 4 and day 14 for all experimental conditions (three CBZ concentrations, two soils, aerobic and saturated conditions). Total genomic DNA extracts were submitted for high throughput amplicon sequencing following the protocol described elsewhere (Caporaso et al., 2012; Caporaso et al., 2011) at the Research Technology Support Facility (RTSF) at Michigan State University. Illumina specific fusion primers were used to amplify the V4 region of the 16S rRNA gene and to add unique barcodes to samples in each well to enable pooling and sequencing. After the amplicons were checked on 1% agarose gel, equimolar amounts of the sample were pooled to normalize results, purified and then sequenced on the Illumina MiSeq™ Personal Sequencing System. The amplicon sequencing data in the fastq file format was

analyzed on Mothur version 1.33.0 from Patrick D. Schloss Laboratory (Schloss, 2009) using the MiSeq standard operating procedure (Schloss, 2013). Barcode information was removed from the sequence data and contiguous sequences were created using the forward and reverse reads, were analyzed for errors and then classified. Samples were checked for the proper read length (<275 bp), ambiguous bases and homopolymer length greater than 8 to eliminate such sequences. These sequences were then aligned with the SILVA bacteria database (Pruesse et al., 2007) for the V4 region. Chimeras, mitochondrial and chloroplast lineage sequences were removed and then the sequences were classified into OTU's. Cho1 and Shannon values were calculated and principal component analyses were performed using Mothur. Illumina sequencing data was deposited in the NCBI Sequence Read Archive under Bioproject: PRJNA311080 and Biosample: SAMN04461763.

To obtain the inferred metagenome from the 16S rRNA gene data, all sequences were first reclassified to the Greengenes gene database (DeSantis et al., 2006) and an OTU biom table was created using Mothur. Following this, normalized OTU tables were generated using PICRUSt (Langille et al., 2013), and these were used to create a functional metagenome prediction file for each sample. The functional metagenome predictions files were analyzed using the "categorize by function" command (level 3) using the KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathways. Only the KEGG pathways concerning the degradation and metabolism of xenobiotic compounds were considered in the data analysis. Following this, the OTUs responsible for contributing to the drug metabolism pathways (cytochrome P450 and other enzymes) were determined using the "metagenome contributions" command in PICRUSt. The drug metabolism pathways were selected for analysis because of the differences between the two soils for these

pathways (see results section). The three OTUs with the highest gene count contribution values for these pathways were determined for soils 1 and 2 under aerobic conditions.

2.3.7. Data Analysis

Statistical analyses included two-tailed t-tests assuming unequal variances. This approach was used to determine if CBZ removal was significant compared to the abiotic controls (soil 1). Further, t-tests were applied to investigate which phylotypes illustrated a difference in relative abundance between the CBZ-amended samples and the live controls (no CBZ added). Finally, the PICRUSt generated data were analyzed to investigate the differences in the abundance of xenobiotic degrading genes between the two soils, at both time points, for the three concentrations under aerobic and anaerobic conditions.

2.4. Results and Discussion

2.4.1. Carbamazepine Biodegradation

The differences in CBZ concentrations between day 4 and 14 were only statistically significant for soil 1. Specifically, under aerobic conditions and under all three concentrations tested, the percent CBZ remaining significantly decreased between days 4 and 14 (Supplementary Table 2.3). The percent decrease under aerobic conditions between these two sampling events was similar at all three concentrations and ranged between 12.8% and 14.5% (Supplementary Figure 2.1A). In contrast, for soil 1, under anaerobic conditions, a statistically significant decrease was observed for only the lowest concentration (p=0.0089). Notably, only duplicates were included for day 14 under anaerobic conditions and this may have influenced the statistical tests. When all samples were considered collectively for all three concentrations under anaerobic conditions, the difference between the two time points was significant (p=0.0261) (Supplementary Table 2.3) and ranged from 6.2% to 14.9%. A follow up experiment with abiotic

controls was completed to determine if the removal in soil 1 was biological. For all treatments tested (aerobic and anaerobic at three CBZ concentrations), there was no statistical difference in percent CBZ remaining between days 4 and 14 in the abiotic controls, indicating the removal in soil 1 was indeed biological (Supplementary Table 2.3).

The results from soil 2 differed considerably from those for soil 1. Although the average CBZ remaining in all treatments was lower at day 14 compared to day 4 (Supplementary Figure 2.1B), the differences were not significant in the majority of the treatments (Supplementary Table 2.3). There was only a statistically significant difference between the two time points for the highest CBZ concentration (5000 ng g^{-1}) (4.8% decrease) under aerobic conditions (p= 0.0314). As limited removal was observed in soil 2, the follow up experiment with abiotic controls was not performed.

The contrasting results from soil 1 and soil 2 may have important implications for the application of biosolids or wastewater effluent to agricultural areas. Soil 2 has high organic matter content (8%) and this could impact the bioavailability of CBZ to the soil microbial community for microbial degradation. On the other hand, the organic matter content of soil 1 was low (1.5%), which may have resulted in CBZ being more readily available for biodegradation. Previous research has indicated a correlation between CBZ sorption and organic matter content (Arye et al., 2011). Therefore, soils with higher organic matter may slow CBZ degradation by limiting bioavailability to soil microorganisms. An alternative hypothesis is that the observed differences in biodegradation results from differences in the functional abilities of the two microbial communities (see PICRUSt data later).

In the current study, for soil 1, the concentration of CBZ did not appear to impact removal trends. In contrast, others have reported no degradation at low CBZ concentrations (50 ng g^{-1}),

but notable degradation at higher concentrations (up to ~25% at 5000 ng g^{-1}) (Grossberger et al., 2014). Conversely, higher initial CBZ concentrations have produced longer half-life values (990 days compared to 138 days for lower CBZ concentrations) in other soil biodegradation experiments (Duran-Alvarez et al., 2015). High CBZ half-life values have also been reported in studies using samples containing environmentally relevant CBZ concentrations. For example, long half-lives were reported in soils amended with untreated wastewater (between 355 and 1624 days) (Dalkmann et al., 2014) and in soils amended with biosolids in an outdoor mesocosm study (between 462 and 533 days) (Walters et al., 2010).

Limited data exist on the susceptibility of CBZ to degradation under anaerobic conditions. Here, the percent CBZ remaining decreased in soil 1 at all three concentrations under anaerobic conditions, but this decrease was only significant at the lowest concentration. Further, no CBZ removal was observed in soil 2 under anaerobic conditions. At least one other study has compared CBZ removal under aerobic and anaerobic conditions. In that research, sediment from three wetlands was examined to evaluate CBZ degradation (Conkle et al., 2012). CBZ half-life values between 207 and 546 days were reported under anaerobic conditions compared to between 165 and 264 days under aerobic conditions. The research presented here, along with the above results, indicates CBZ is more recalcitrant under anaerobic conditions and may therefore persist longer in saturated soils.

An additional set of experiments was performed to determine the long term biodegradation potential of CBZ in soil 1 at the lowest concentration (50 ng g^{-1}) under both aerobic and anaerobic conditions (Supplementary Figure 2.2). CBZ biodegradation under aerobic conditions was only observed at the last sampling point (day 50, p=0.005 for a t-test between control and samples). Further, no removal was observed under anaerobic conditions. The increased time

required for CBZ degradation under aerobic conditions and the lack of degradation under anaerobic conditions can likely be attributed to soil storage time, as the experiment was performed one year after the short term experiment.

2.4.2. Impact of CBZ on Microbial Community

A summary of the analyzed sequence data is shown (Supplementary Table 2.4). The total number of OTUs or species richness was estimated using the Chao1 estimator (Supplementary Table 2.5). Soil 2 appeared to have greater species richness compared to soil 1. Also, species richness was lower under anaerobic conditions compared to aerobic conditions for both soils. There was only a statistically significant difference in species richness for the samples compared to the live controls (no CBZ added) in the those amended with 500 ng g^{-1} CBZ in soil 1 at day 14 and soil 2 at day 4 (both aerobic conditions).

The calculated Shannon diversity values were also generally higher for soil 2 compared to soil 1 (Supplementary Table 2.6). There was a statistically significant decrease in the diversity values between the CBZ-amended samples for soil 1 (at all three concentrations) and the controls at day 14. In contrast, under anaerobic conditions, there was a statisitically significant increase in the diversity values between the CBZ-amended samples for soil 1 (higher two concentrations) and the controls at day 14.

The sequencing data were classified into phyla to determine the overall microbial diversity in the samples and CBZ-free controls for both soils. In soil 1, under aerobic and anaerobic conditions, the most dominant phyla were *Proteobacteria*, followed by *Bacteroidetes, Acidobacteria, Verrucomicrobia* and *Actinobacteria* (Supplementary Figure 2.3). In this soil, the abundance of several phyla was impacted by the presence of CBZ. In three of the four treatments (two time points, aerobic and anaerobic conditions) studied, *Actinobacteria* and *Bacteroidetes*

were significantly impacted. In two of the four treatments, the phyla *WS3* and *Gemmatimonadetes* were affected. In one of the four treatments, other impacted phyla included *BRC1, Spirochaetes, Chloroflexi, Nitrospira* and *Proteobacteria*.

Similar phyla were present in soil 2 compared to soil 1 (Supplementary Figure 2.4). *Proteobacteria* was also the most abundant, but in this case, *Acidobacteria* was the second most abundant. The third most abundant was *Bacteroidetes*, followed by *Verrucomicrobia* and then *Actinobacteria.* Similar to soil 1, the abundance of *Bacteroidetes* was significantly different in the CBZ-amended samples compared to the controls in two of the four treatments (aerobic day 4 and 14). Other phyla were only impacted in one of the four treatments and these included *Actinobacteria, Acidobacteria, WS3, Chloroflexi, Gemmatimonadetes, Chloroflexi* and *Proteobacteria*. Principal component analysis was performed on the sequencing data. Only one dataset exhibited significant clustering for the CBZ-amended samples compared to the controls and the initial samples (soil 1, aerobic conditions at day 14) (Supplementary Figure 2.5).

2.4.3. Identifying Putative Carbamazepine Degrading Phylotypes

The microorganisms putatively linked to CBZ degradation were only investigated for soil 1, as there was limited removal in soil 2. The aim was to determine which phylotypes increased in abundance following CBZ biodegradation, and could therefore be putatively associated with CBZ degradation. These microorganisms could be using CBZ as a carbon, nitrogen or an energy source. For this, percent relative abundance values were compared between CBZ-amended microcosms and live control microcosms (CBZ-free controls) to determine which phylotypes increased in abundance. Specifically, t-tests (two-tailed, unequal variance) were performed to determine which phylotypes were more abundant in the CBZ-amended samples compared to the live controls.

In soil 1, at day 4, under aerobic conditions, no phylotypes statistically significantly increased in abundance in the CBZ-amended samples compared to the live controls. However, by day 14, many phylotypes were more abundant in the CBZ-amended samples compared to the controls. In these experiments, 44, 17 and 22 phylotypes were enriched in the samples amended with 50, 500 and 5000 ng g^{-1} CBZ, respectively (data not shown). From these, 16 phylotypes were enriched in two or more of the concentrations tested (Figure 2.1). The highest levels of enrichment were noted for unclassified *Sphingomonadaceae*, unclassified *Xanthomonadaceae* and *Sphingomonas*. Only three phylotypes were enriched at all three concentrations, including *Aquicella*, *Microvirga* and unclassified *Rhodobacteraceae*. These enrichment patterns indicate these specific phylotypes are benefiting from CBZ degradation and are likely CBZ degraders.

It is difficult to compare the above findings concerning unclassified *Sphingomonadaceae* and unclassified *Xanthomonadaceae* to previous research, as in the current work, the specific genera responsible were not determined. However, many studies have illustrated the ability of *Sphingomonas* spp. to degrade organic environmental contaminants. For example, just within the past couple of years, microorganisms in the genus *Sphingomonas* spp. have been linked to the degradation of dibenzofuran (Coronado et al., 2015), phenanthrene (Pan et al., 2016), azo dyes (Nadh et al., 2015), the fungicide ortho-phenylphenol (Perruchon et al., 2016), the chloroacetamide herbicides, butachlor, acetochlor and alachlor (Chen et al., 2015) and bisphenol-A (Matsumura et al., 2015). The data suggest that *Sphingomonas* are important for the biodegradation of many organic chemicals and, as shown here, are likely also involved in CBZ biodegradation.

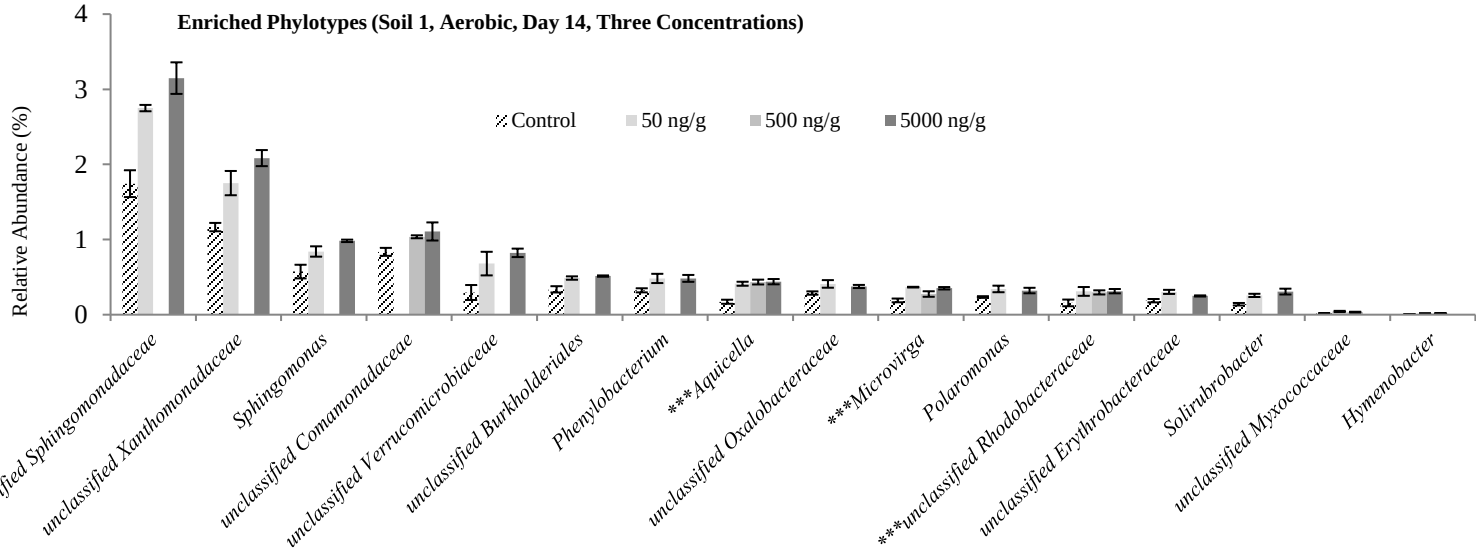

Figure 2. 1 Common enriched phylotypes ($p < 0.05$) in at least two CBZ-amended treatments (50 ng g-1, 500 ng g-1 and 5000 ng g-1 CBZ) compared to the controls in the aerobic treatments at day 14 (soil 1). Three asterisks indicate the phylotypes enriched ($p < 0.05$) at all three concentrations. The bars represent standard deviations from triplicate microcosms. No phylotypes were enriched at day 4.

Limited information exists on the genus *Aquicella* (*Gammaproteobacteria, Legionellales, Coxiellaceae*). Two novel isolates were obtained from well and spa water in central Portugal (Santos et al., 2003) and sequences classifying as *Aquicella* were reported in rhizospheres of field-grown potato plants (Kobayashi et al., 2015). However, previous research has documented that *Aquicella* illustrated a positive response in soils amended with phenanthrene (Ding et al., 2012). Microorganisms in the genus *Microvirga* (*Alphaproteobacteria, Rhizobiales, Methylobacteriaceae*) are symbiotic nitrogen-fixing bacteria (Ardley et al., 2012; Radl et al., 2014; Reeve et al., 2014) and have also been isolated from free-flowing geothermal waters (Kanso and Patel, 2003). Notably, microorganisms classifying as *Microvirga* were found in a biofilm exposed to crude oil, n-hexadecane and phenanthrene contaminated sewage effluent (Al-Mailem et al., 2014). Microorganisms in the family *Rhodobacteraceae* have been found in coastline water samples exposed to crude oil (Acosta-Gonzalez et al., 2015) and have been associated with the degradation of different polyaromatic hydrocarbon (PAH) compounds (Gutierrez et al., 2011; Harwati et al., 2009; Pinyakong et al., 2012; Syakti et al., 2013). The results presented here, along the studies discussed above, suggest similarities in the phylotypes able to degrade PAH and CBZ. This pattern is perhaps not surprising, as CBZ contains two aromatic ring moieties.

In soil 1, under anaerobic conditions, there was only a statistically significant difference between CBZ remaining at the two time points at the lowest CBZ concentration (Supplementary Table 2.3). However, when the data were combined from all three concentrations, a significant decrease between the two time points was observed (p=0.0261). Therefore, the phylotypes that increased in abundance in CBZ treated samples were compared to the live CBZ-free controls for the anaerobic experiments. At the early time point (day 4), 5, 14 and 5 phylotypes were enriched

in the CBZ-amended microcosms compared to the controls at 50, 500 and 5000 ng g^{-1}, respectively (Supplementary Figure 2.6). Only three phylotypes were enriched in more than one of the concentrations tested, included unclassified *Desulfuromonadaceae*, *Pusillimonas* (both with relative abundances of < 0.009%) and *Armatimonadetes gp5* (relative abundance of > 0.5%). Very little is known about the genus *Armatimonadetes gp5* (phylum *Armatimonadetes*) and, according to the Ribosomal Database Project, the genus contains only 14 uncultured microorganisms. The data generated in the current study indicate a potentially significant role of this phylotype in CBZ degradation. The low relative abundance values of the other two phylotypes suggests their roles are likely minor.

A more limited effect on the microbial community was noted for soil 1, under anaerobic conditions, at the later time point (day 14). For this, only one phylotype was enriched at 50 and 5000 ng g^{-1} and nine phylotypes were enriched at 500 ng g^{-1} (Supplementary Figure 2.7). No phylotypes were enriched in more than one concentration and only two phylotypes (*Cryobacterium* and *Solirubrobacter*) were enriched at more than 0.3% relative abundance (Supplementary Figure 2.7B, C). These weak trends make it difficult to link specific phylotypes with CBZ removal under anaerobic conditions.

As stated above, only *Rhodococcus rhodochrous*, *Aspergillus niger* and *Streptomyces* MIUG 4.89 have been previously linked to CBZ degradation (Gauthier et al., 2010; Popa et al., 2014). Interestingly, no enrichment of these genera was observed here following CBZ degradation, suggesting that pure culture research may inaccurately portray the microorganisms responsible for CBZ in soil communities when exposed to environmentally relevant CBZ concentrations.

2.4.4. PICRUSt Analysis

PICRUSt was used to predict the functional composition of the metagenomes from soils 1 and 2 under the different treatments and time points. The analysis focused on the genes associated with xenobiotic degradation pathways. The percent predicted abundance of these genes was compared between the soils. Under aerobic conditions, there was a statistically significant difference between the two soils for several pathways, including DDT degradation, atrazine degradation, chlorocyclohexane and chlorobenzene degradation, drug metabolism-other enzymes, drug metabolism- cytochrome P450, fluorobenzoate degradation and nitrotoluene degradation (Table 2.1). For the majority of these pathways (except nitrotoluene degradation), the genes were more abundant in soil 1 compared to soil 2 in most of the DNA extracts examined (in bold in Table 2.1). The trends were less pronounced under anaerobic conditions, which involved a statistically significant difference for three pathways (chlorocyclohexane and chlorobenzene degradation, drug metabolism-other enzymes, and nitrotoluene degradation) (Table 2.2). Two of the three pathways (except nitrotoluene) were more important in soil 1. The drug metabolism pathways were selected for further analysis using PICRUSt because of the consistent difference between the two soils for these pathways. Specifically, the analysis involved the identification of the phylotypes in soils 1 and 2 (aerobic conditions) linked to the KEGG entries in the drug metabolism pathways (other enzymes and cytochrome P450) (Supplementary Tables 2.7 and 2.8). The phylotypes contributing to the high gene counts for these KEGG entries were summarized for soil 1 (Table 2.3). The most important microorganisms in soil 1 contributing to the drug metabolism KEGG entries include unclassified *Chitinophagaceae*, unclassified *Saprospiraceae*, Ellin6075 (phylum *Acidobacteria*), *Kaistobacter* (family *Sphingomonadaceae*) and *Arenimonia oryziterrae* (family

Xanthomonadaceae). It is interesting to note that both *Sphingomonadaceae* and *Xanthomonadaceae* were again identified as being implicated in drug metabolism.

Overall, the PICRUSt data support the experimental data described above, that is 1) biodegradation potential was more important in soil 1 compared to soil 2 and 2) biodegradation was more likely to occur under aerobic conditions. It would be interesting to apply this analysis method to other samples, to determine if this approach can predict CBZ biodegradation in other agricultural soils or in WWTP samples.

Table 2. 1 T-test (two tailed, unequal variance) comparison of xenobiotic degradation pathways (generated from PICRUSt) between soil 1 and 2 under aerobic conditions. Those significantly different in 4 or more from the 6 comparisons are shown in bold. Note: NS - not significant.

	50 - Day 4		**50 - Day 14**		**500 - Day 4**		**500 - Day 14**		**5000 - Day 4**		**5000 - Day 14**	
	p value	**trend**	**p value**	**trend**	**p value**	**trend**	**p value**	**trend**	**p value**	**trend**	**p value**	**trend**
Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)	**0.006**	**1 > 2**	**0.010**	**1 > 2**	**0.001**	**1 > 2**	**0.008**	**1 > 2**	**0.012**	**1 > 2**	**0.043**	**1 > 2**
Aminobenzoate	> 0.05		0.015	1 > 2	NS		NS		NS		0.026	1 > 2
Atrazine	**NS**		**0.002**	**2> 1**	**0.040**	**2 > 1**	**0.039**	**2 > 1**	**NS**		**0.002**	**2 > 1**
Benzoate	NS		0.005	1 > 2	NS		NS		NS		0.015	1 > 2
Bisphenol	NS		0.038	1 > 2	NS		NS		NS		NS	
Caprolactam	NS		0.029	1 > 2	NS		NS		NS		NS	
Chloroalkane & chloroalkene	NS		0.001	1 > 2	0.013	1 > 2	0.044	1 > 2	NS		0.002	1 > 2
Chlorocyclohexane & chlorobenzene	**NS**		**0.000**	**1 > 2**	**0.005**	**1 > 2**	**0.004**	**1 > 2**	**0.015**	**1 > 2**	**0.000**	**1 > 2**
Dioxin	NS		0.003	1 > 2	NS		NS		NS		0.034	1 > 2
Drug metabolism - cytochrome P450	**NS**		**0.001**	**1 > 2**	**0.001**	**1 > 2**	**0.048**	**1 > 2**	**NS**		**0.012**	**1 > 2**
Drug metabolism - other enzymes	**0.003**	**1 > 2**	**0.001**	**1 > 2**	**0.000**	**1 > 2**	**0.000**	**1 > 2**	**0.001**	**1> 2**	**0.002**	**1 > 2**
Ethylbenzene	NS		0.003	1 > 2	0.034	1 > 2	NS		NS		0.004	1 > 2
Fluorobenzoate	**NS**		**0.000**	**1 >2**	**0.034**	**1 > 2**	**0.007**	**1 >2**	**0.035**	**1 >2**	**0.000**	**1 >2**
Metabolism of xenobiotics by cytochrome P450	NS		0.002	1 > 2	0.002	1 > 2	NS		NS		0.020	1 > 2
Naphthalene	NS		0.012	1 > 2	NS		NS		NS		0.020	1 > 2
Nitrotoluene	**0.021**	**2 > 1**	**0.000**	**2 > 1**	**0.001**	**2 > 1**	**0.002**	**2 > 1**	**0.018**	**2 > 1**	**0.008**	**2 > 1**
Polycyclic aromatic hydrocarbon	NS		NS		NS		NS		NS		NS	
Styrene	NS		0.002	1 > 2	0.017	1 > 2	NS		NS		0.001	1 > 2
Toluene	NS		0.002	1 > 2	NS		0.006	1 > 2	NS		0.000	1 > 2
Xylene	NS		0.004	1 > 2	NS		NS		NS		0.003	1 > 2

Table 2. 2 T-test (two tailed, unequal variance) comparison of xenobiotic degradation pathways (generated from PICRUSt) between soil 1 and 2 under anaerobic conditions. Those significantly different in 4 or more from the 6 comparisons are shown in bold. Note: NS - not significant.

	50 - Day 4		**50 - Day 14**		**500 - Day 4**		**500 - Day 14**		**5000 - Day 4**		**5000 - Day 14**	
	p value	**trend**	**p value**	**trend**	**p value**	**trend**	**p value**	**trend**	**p value**	**trend**	**p value**	**trend**
Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)	0.026	1 > 2	NS		0.017	1 > 2	NS		NS		NS	
Aminobenzoate	NS		NS		NS		NS		NS		NS	
Atrazine	NS		NS		0.023	2 > 1	NS		NS		NS	
Benzoate	0.021	1 > 2	NS		NS		NS		NS		NS	
Bisphenol	NS		NS		NS		NS		NS		NS	
Caprolactam	NS		0.043	2 > 1	NS		NS		NS		NS	
Chloroalkane & chloroalkene	**0.001**	**1 > 2**	**0.039**	**1 > 2**	**0.008**	**1 > 2**	**0.038**	**1 > 2**	**NS**		**NS**	
Chlorocyclohexane & chlorobenzene	0.001	1 > 2	NS		0.006	1 > 2	0.014	1 > 2	NS		NS	
Dioxin	NS		0.035	1 > 2	NS		NS		NS		NS	
Drug metabolism - cytochrome P450	0.008	1 > 2	NS		0.002	1 > 2	0.001	1 > 2	NS		NS	
Drug metabolism - other enzymes	**0.001**	**1 > 2**	**0.041**	**1 > 2**	**0.008**	**1 > 2**	**0.024**	**1 > 2**	**NS**		**NS**	
Ethylbenzene	0.001	1 > 2	0.008	1 > 2	0.009	1 > 2	NS		NS		NS	
Fluorobenzoate	NS		NS		NS		0.048	1 > 2	NS		NS	
Metabolism of xenobiotics by cytochrome P450	0.016	1 > 2	NS		0.007	1 > 2	0.002	1 > 2	NS		NS	
Naphthalene	0.008	1 > 2	0.040	1 > 2	0.028	1 > 2	NS		NS		NS	
Nitrotoluene	**0.000**	**2 > 1**	**0.001**	**2 > 1**	**0.022**	**2 > 1**	**0.043**	**2 > 1**	**NS**		**0.009**	**2 > 1**
Polycyclic aromatic hydrocarbon	NS		NS		NS		0.017	2 > 1	NS		NS	
Styrene	0.006	1 > 2	NS		0.021	1 > 2	NS		NS		NS	
Toluene	NS		0.042	1 > 2	NS		NS		NS		NS	
Xylene	0.007	1 > 2	NS		0.038	1 > 2	NS		NS		NS	

Table 2. 3 The OTUs in soil 1 primarily responsible for the KEGG entries for the drug metabolism pathways. Values are gene counts (average and standard deviations for triplicate samples) contributed by each OTU. See Supplementary Tables 7 and 8 for the complete lists.

	Day 4		Day 14		Operational Taxonomic Unit
KEGG Entry (Drug metabolism - other enzymes)	**Average**	**Stdev**	**Average**	**Stdev**	
Uridine phosphorylase (K00757)	1232	170	1329	202	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	1262	147	1101	205	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
Uridine kinase (K00876)	2062	225	2327	472	*Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified*
	1262	147	1101	205	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
Thymidine kinase (K00857)	2062	225	2327	472	*Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified*
	1232	170	1329	202	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	1232	147	1101	205	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
Hypoxanthine phosphoribosyltransferase (K00760)	2062	225	2327	472	*Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified*
	1213	244	1728	157	*Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae, Candidatus Solibacter, unclassified*
	1232	170	1329	202	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
Cytidine deaminase (K01489)	2062	225	2327	472	*Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified*
	1235	170	1329	202	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	1259	147	1101	205	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
Beta-glucuronidase (K01195)	2062	225	2327	472	*Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified*
	1232	170	1329	202	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
GMP synthase (glutamine-hydrolysing) (K01951)	2062	225	2327	472	*Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified*
	1213	198	1546	384	*Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Kaistobacter, unclass.*
	1378	221	1280	224	*Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Arenimonas, oryziterrae*
IMP dehydrogenase (K00088)	2062	225	2327	472	*Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified*
	1232	170	1329	202	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	1262	147	1101	205	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
Uridine monophosphate synthetase (K13421)	1262	147	1101	205	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
	406	114	509	147	*Chloroflexi, Anaerolineae, SBR1031, A4b, unclassified*
Thiopurine S-methyltransferase (K00569)	1262	147	826	205	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
	689	110	640	112	*Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Arenimonas, oryziterrae*
KEGG Entry (drug metabolism - cytochrome P450)					
Monoamine oxidase (K00274)	2464	340	2658	403	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	2062	225	2327	472	*Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified*
	1262	147	1101	205	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
Glutathione S-transferase (K00799)	5457	891	6958	1727	*Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Kaistobacter,*
	5457	901	6242	198	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Rhodoplanes, unclass.*

2.4.5. Phylotypes Negatively Impacted by Carbamazepine Exposure

A secondary objective of this study was to determine if exposure to CBZ negatively affected specific phylotypes in the soil microbial community. For this, the relative abundance of phylotypes in the CBZ-amended samples was compared to the live CBZ-free controls to determine if any significantly (p<0.05) decreased in abundance because of the addition of CBZ. This analysis was performed in soils 1 and 2, under both aerobic and anaerobic conditions.

In soil 1, under aerobic conditions, at day 4, four and two phylotypes decreased in abundance at 50 ng g^{-1} and 5000 ng g^{-1}, respectively, and these were different for each treatment (Figure 2.2A and 2.2B). The difference in relative abundance, compared to the controls, was greatest at 5000 ng g^{-1} (~0.3% for *Geobacter* and *Flavobacterium*). At the 500 ng g^{-1} treatment level, no effect was noted for any phylotype. These data indicate a limited effect of CBZ on the soil microbial community for this short time period. In contrast, in soil 1, by day 14 (also under aerobic conditions) a much greater effect was observed following exposure to CBZ. The greatest decrease, compared to the controls, was noted for unclassified *Bacteria* (1.3%), unclassified *Bacteroidetes* (1.5%) and *3 genus incertae sedis* (2.2%). Many common phylotypes decreased in abundance compared to the controls at all three concentrations (Figure 2.2C). These included

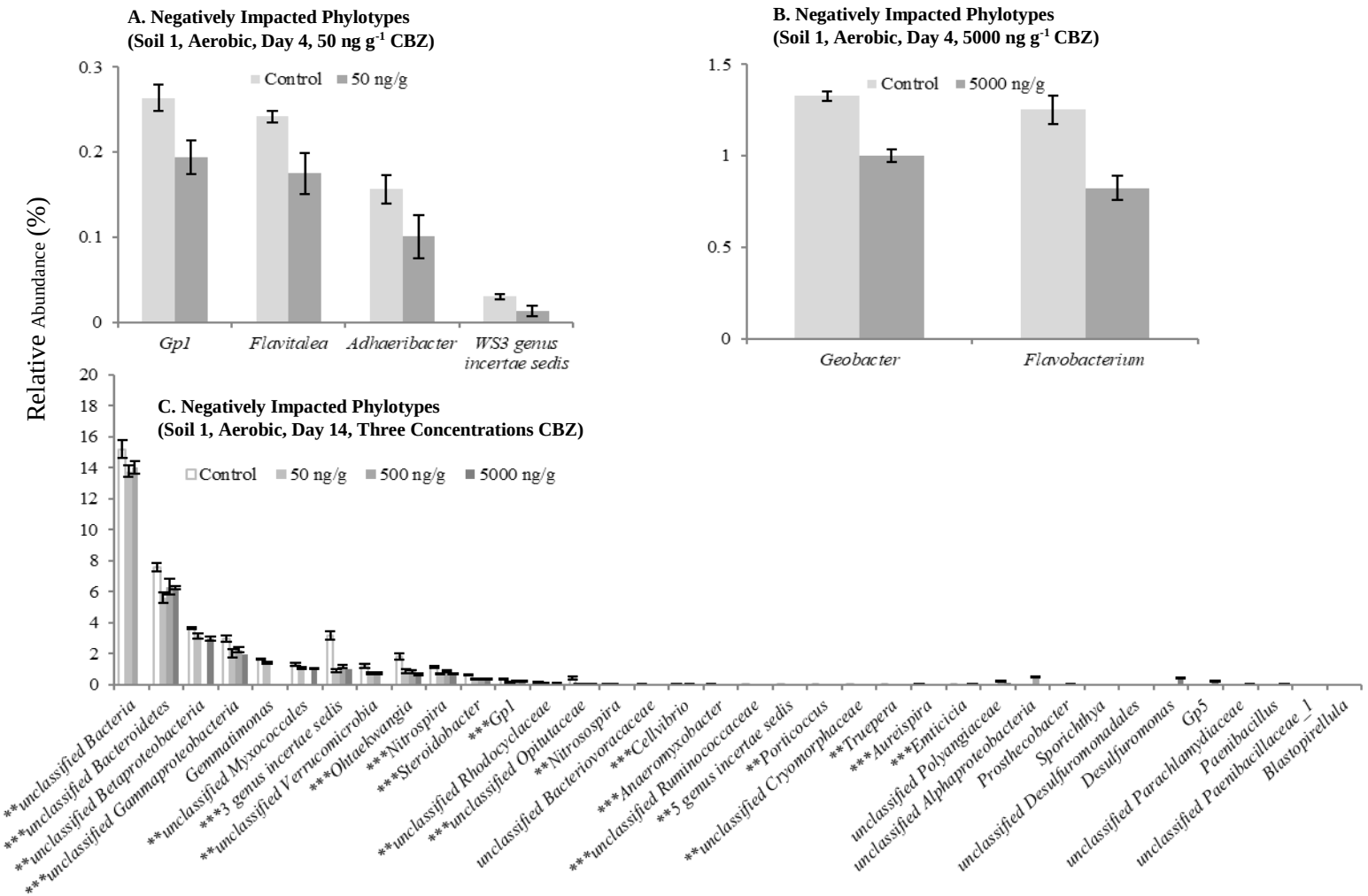

Figure 2.2 Phylotypes that decreased in abundance in the CBZ-amended samples compared to the controls ($p < 0.05$) in soil 1 at 50 ng g-1 (A) and 5000 ng g-1 CBZ (B) in the aerobic treatments at day 4 (no differences were noted for 500 ng g-1). The phylotypes that significantly decreased at the three concentrations at day 14 are also shown (C). Two and three asterisks indicate the phylotypes decreased in abundance at two and three concentrations, respectively. The bars represent standard deviations from replicates or triplicates.

unclassified *Bacteroidetes*, unclassified *Gammaproteobacteria, 3 genus incertae sedis, Ohtaekwangia, Nitrospira, Steroidobacter, Gp1, unclassified Opitutaceae, Cellvibrio, Anaeromyxobacter, unclassified Ruminococcaceae, Aureispira* and *Emticicia*. In addition, a further ten phylotypes decrease in abundance in two of the three concentrations. These data imply that a longer time period is needed for CBZ to significantly affect the microbial community.

In soil 1, under anaerobic conditions at day 4 and 14, a number of phylotypes were also negatively affected by exposure to CBZ (Figure 2.3). At day 4, under 12 phylotypes decreased in abundance at each treatment level (Figure 2.3A, B, C). From these, three microorganisms (unclassified *Polyangiaceae, Rhizobium* and *Armatimonadetes gp4*) decreased in abundance in at least two of the treatments. The difference in relative abundance, compared to the controls, was greatest for *Methylophilus* (~3%) (Figure 2.3A). By day 14, a smaller number of phylotypes were affected (6 or less) and two different microorganisms (*Iamia* and *Luteibacter*) decreased in abundance in at least two of the treatment concentrations (Figure 2.3D, E, F).

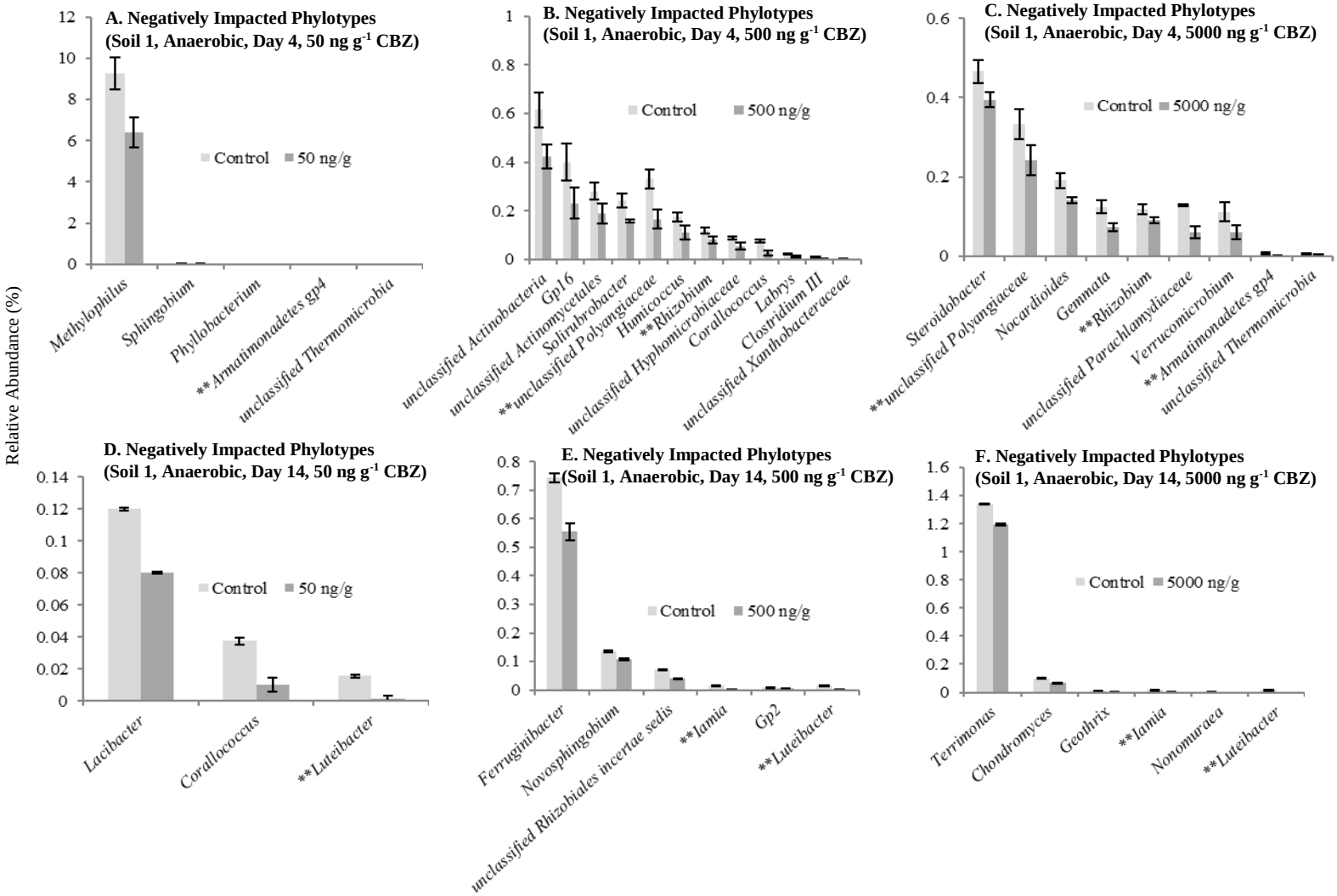

Figure 2.3 Phylotypes that decreased in abundance in the CBZ-amended samples compared to the controls ($p < 0.05$) in soil 1 at 50 ng g-1 (A, D), 500 ng g-1 (B, E) and 5000 ng g-1 CBZ (C, F) in the anaerobic treatments at day 4 (A, B, C) and 14 (D, E, F). Two asterisks indicate the phylotypes decreased in abundance in at least two concentrations. The bars represent standard deviations from replicates or triplicates.

In soil 2, under aerobic conditions, the effect of CBZ on the soil microbial community was more pronounced at day 4. In this soil, an effect was noted at all three concentrations at day 4 and up to eight phylotypes were impacted in any one treatment (Figure 2.4A, B C). Three phylotypes were commonly impacted in at least two of the concentrations tested, including *unclassified Chitinophagaceae*, *Aureispira* (also affected in soil 1) and *Paenibacillus*. Similar to soil 1 under aerobic conditions, *Flavobacterium* illustrated a decrease in relative abundance compared to the controls (Figure 2.4A). By day 14, only two phylotypes were affected by the presence of CBZ.

However, one phylotype, unclassified *Bacteroidetes*, illustrated a large decrease in abundance at all three concentrations (~1%) (Figure 2.4D). This phylotype was also impacted in soil 1 (under aerobic conditions). Similar to the data from day 4, unclassified *Chitinophagaceae* also exhibited a decrease in abundance at day 14 (for 50 ng g^{-1} only) (Figure 2.4D).
The difference between the effect of CBZ under aerobic conditions between soil 1 and soil 2 may be explained by a decreased bioavailability in soil 2 due to increased sorption to soil organic matter (higher % organic matter). In soil 1, many phylotypes declined in abundance by day 14, however, in soil 2, only two decreased in abundance by this time.

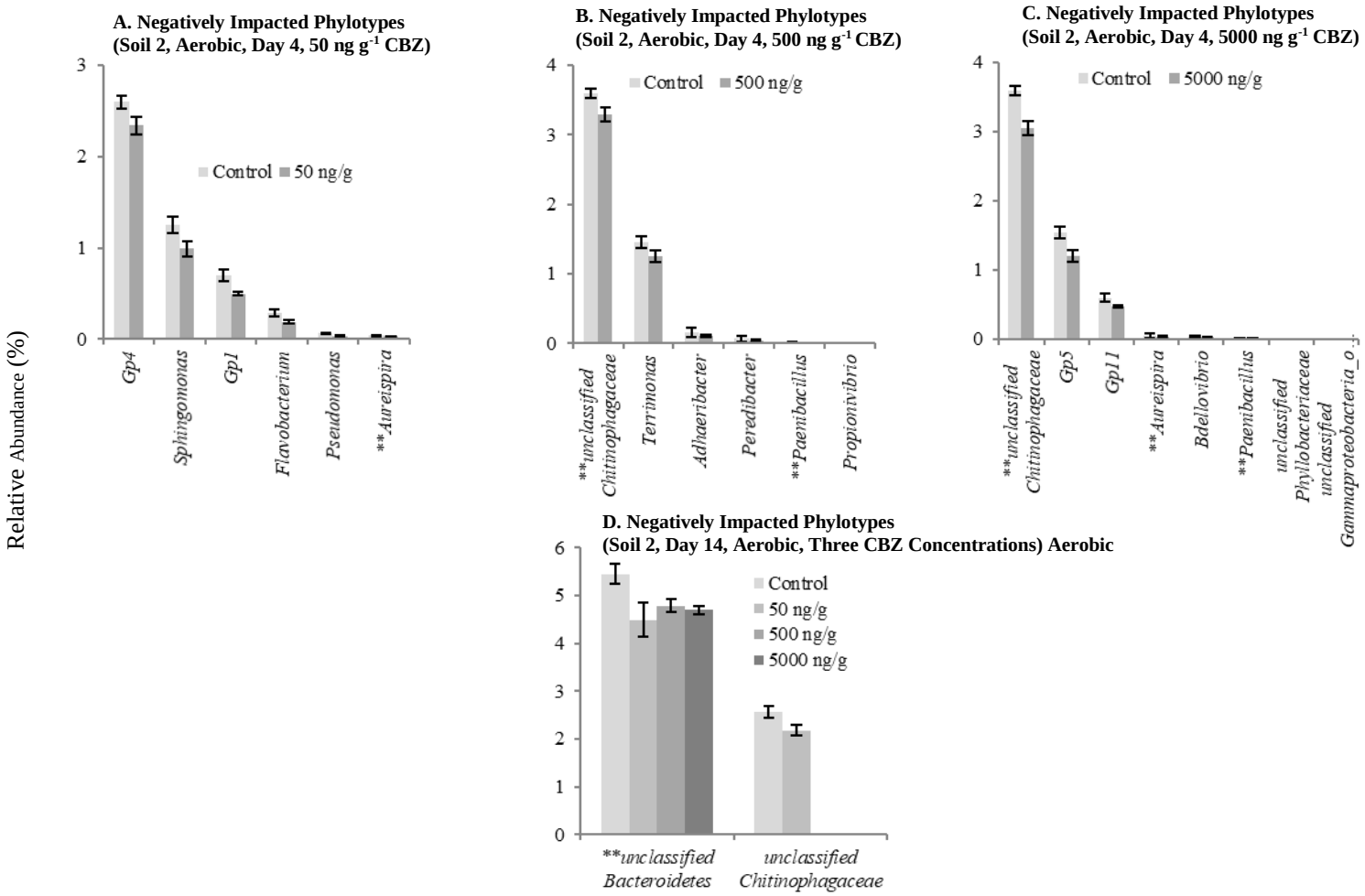

Figure 2.4 Phylotypes that decreased in abundance in the CBZ-amended samples compared to the controls ($p < 0.05$) in soil 2 at 50 ng g-1 (A), 500 ng g-1 (B) and 5000 ng g-1 CBZ (C) in the aerobic treatments at day 4 (A, B, C) and 14 (D). Two asterisks indicate the phylotypes decreased in abundance in at least two concentrations. The bars represent standard deviations from replicates or triplicate microcosms.

In soil 2, under anaerobic conditions, different microorganisms were impacted by CBZ. Between three and ten microorganisms decreased in abundance compared to the controls at the three concentrations investigated (Figure 2.5). At day 4, only two, *Pasteuria* and unclassified *Bacilli*, were impacted in more than one treatment (Figure 2.5A, B, C). Similar to soil 1 (aerobic conditions) and soil 2 (aerobic conditions), *Flavobacterium* again exhibited a decrease in abundance compared to the controls (Figure 2.5A). By day 14, fewer phylotypes (three or less for each treatment) were influenced by the presence of CBZ. Only one, *Lewinella*, decreased in abundance compared to the controls in more than one of the treatments (Figure 2.5D, E). Interestingly, similar to soil 1, the phylotype *3 genus incertae sedis* illustrated a large decrease (~0.8%) compared to the control at day 14 (Figure 2.5F).

The implications of these findings on ecosystem function are uncertain, but it is clear that the presence of CBZ does impact the microbial community. In many cases, the differences in relative abundances between the CBZ-free controls and the CBZ-amended samples were small. However, for other phylotypes, including *Methylophilus, Lacibacter, Geobacter, Flavobacterium*, 3 *genus incertae sedis, Gp4*, unclassified *Chitinophagaceae* and unclassified *Bacteroidetes*, the percent decrease was considerably higher. Further, for several phylotypes the relative abundance was reduced in both soils (*Flavobacterium*, 3 *genus incertae sedis* and unclassified *Bacteroidetes*), indicating a notable sensitivity to CBZ for these microorganisms.

As two of the phylotypes listed above could not be classified to the genus level (unclassified *Chitinophagaceae* and unclassified *Bacteroidetes*) it is difficult to predict the effect of their reduced population on the function of the soil community.

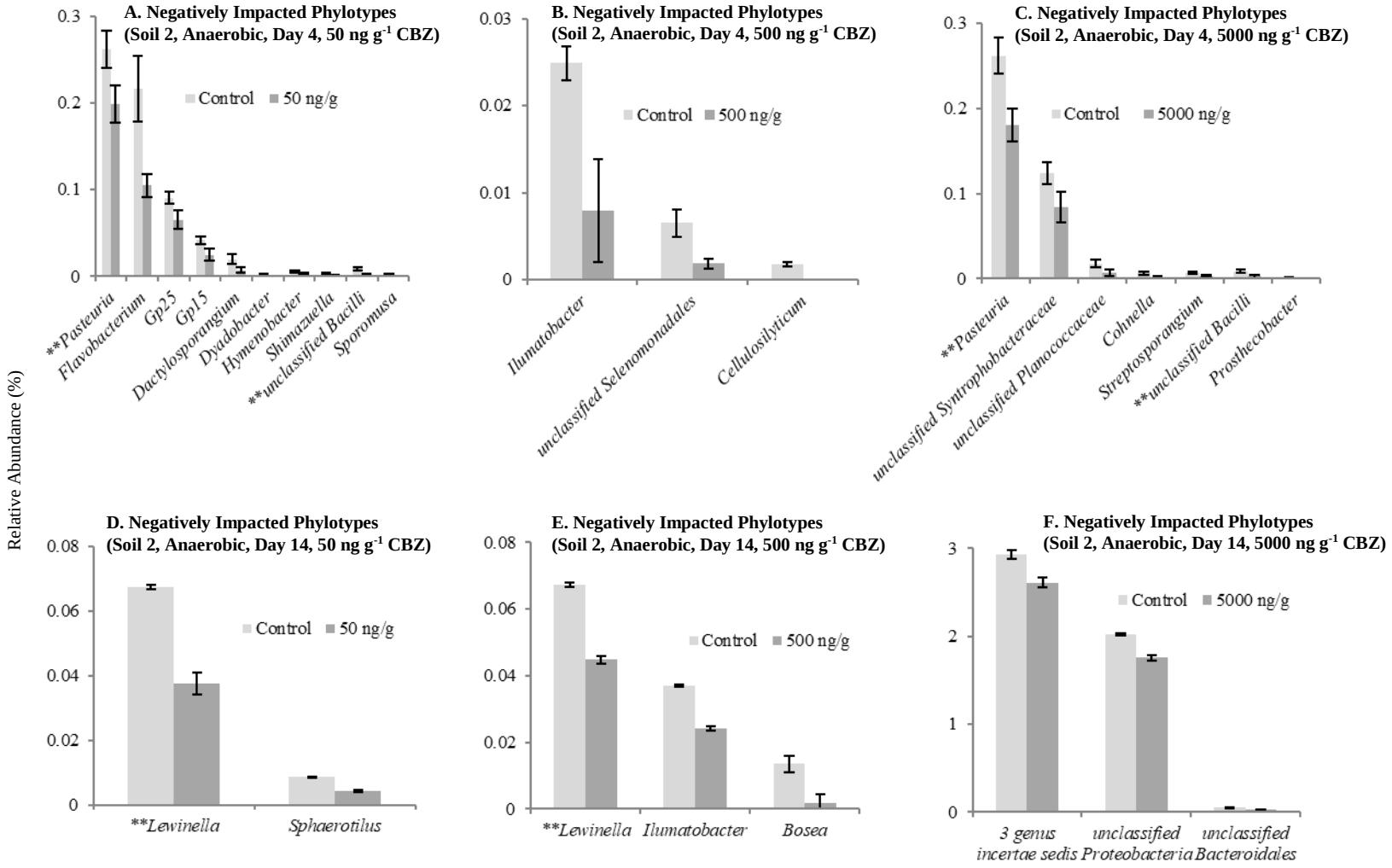

Figure 2.5 Phylotypes that decreased in abundance in the CBZ-amended samples compared to the controls ($p < 0.05$) in soil 2 at 50 ng g-1 (A, D), 500 ng g-1 (B, E) and 5000 ng g-1 CBZ (C, F) in the anaerobic treatments at days 4 (A, B, C) and 14 (D, E, F). Two asterisks indicate the phylotypes decreased in abundance in at least two concentrations. The bars represent standard deviations from replicates or triplicates.

The family *Chitinophagaceae* (phylum *Bacteroidetes*) contains several genera, including *Lacibacter* (also impacted), *Balneola, Filimonas, Flavisolibacter, Gracilimonas, Niastella, Terrimonas* and the type genus *Chitinophaga* (Rosenberg, 2014).

Some species can degrade chitin and the hydrolysis of cellulose is also associated with some species (Rosenberg, 2014). Therefore, it is possible that a reduction in the population of this family could impact soil carbon cycling. It is impossible predict the effect of a reduction in unclassified *Bacteroidetes*, as this phylum contains many genera. The decreased relative abundance of *Methylophilus* may also have implications for carbon cycling, as this genus has been associated with methanol utilization (Xia et al., 2015; Yomantas et al., 2010). *Flavobacterium* (phylum *Bacteroidetes*) are chemoorgantrophic and so their decreased abundance could also influence the carbon cycle. At least one isolate from this genus has been associated with pesticide degradation (Nayarisseri et al., 2015). The impact on the *Geobacter* population may influence iron cycling in soils, as a key characteristic of *Geobacter* is iron reduction (Zacharoff et al., 2016).

The phylotype 3 *genus incertae sedis* belongs to the class *Subdivision 3,* within the phylum *Verrucomicrobia*. Bacteria belonging to this phylum are commonly present in surface soils, however they are typically difficult to cultivate (Bergmann et al., 2011; Janssen, 2006; Janssen et al., 1997; Joseph et al., 2003). The ecology of this phylum remains poorly understood (Bergmann et al., 2011) and hence it is difficult to predict the effect of a reduced population on soil function. CBZ may also impact other functions associated with microorganisms for which little information exists, e.g. *Gp4*.

2.5. Conclusions

CBZ biodegradation was observed in soil 1, but not in soil 2, within the time period investigated. In soil 1, under aerobic conditions, the percent CBZ decrease between days 4 and 14 was similar at all three concentrations. In contrast, for soil 1, CBZ biodegradation was less consistent under anaerobic conditions, indicating CBZ will likely be more recalcitrant under water saturated and oxygen depleted conditions. In soil 1, many phylotypes were more abundant in the CBZ-amended samples compared to CBZ-free controls. Higher levels of enrichment were noted for unclassified *Sphingomonadaceae*, unclassified *Xanthomonadaceae* and *Sphingomonas*. Only three phylotypes were enriched at all three concentrations, including *Aquicella*, *Microvirga* and unclassified *Rhodobacteraceae*. The enrichment patterns indicate these specific phylotypes are benefiting from CBZ degradation. The results presented here, along with previous research on these microorganisms, suggest similarities in the phylotypes able to degrade PAHs and CBZ. The current research did not observe an enrichment of any of the three isolates previously linked to CBZ degradation. These trends suggest that pure culture research may inaccurately portray the microorganisms responsible for CBZ degradation in soil communities. Using PICRUSt, the genes associated with drug metabolism were more important in soil 1 compared to soil 2 and these were also linked to unclassified *Sphingomonadaceae*, unclassified *Xanthomonadaceae*. These results lend support to the hypothesis that the difference in CBZ biodegradation capacity between the two soils is, in part, a result of differences in the microbial communities. This work offers a potentially useful platform to study CBZ biodegradation potential in other soils.

A secondary objective was to determine if exposure to CBZ negatively affected specific phylotypes in the soil microbial community. For several phylotypes, including *Methylophilus, Lacibacter, Geobacter, Flavobacterium*, 3 *genus incertae sedis, Gp4,* unclassified

Chitinophagaceae and unclassified *Bacteroidetes*, a particularly notable decrease in relative abundance was observed in the CBZ-amended samples compared to the controls. For three of these phylotypes (*Flavobacterium*, 3 *genus incertae sedis* and unclassified *Bacteroidetes*), the relative abundance was reduced in both soils, indicating a notable sensitivity to CBZ for these microorganisms. The observed impact of CBZ on some phylotypes may have implications for carbon cycling, pesticide degradation or iron reduction in soils.

In summary, this is the first study to identify putative CBZ degraders in soil communities. The results are particularly important because environmental relevant CBZ concentrations were examined. In addition, this is the first report on the effect of CBZ on specific soil phylotypes. Further, the work suggests that insights into CBZ biodegradation potential can be obtained from examining the abundance of xenobiotic degrading genes using PICRUSt.

APPENDIX

Appendix

Supplementary Table 2.1 Select physical chemical properties of the two soils.

Physical Chemical Properties	Soil 1	Soil 2
Sand (%)	82	58
Silt (%)	12	30
Clay (%)	6	12
Textural classification	Loamy sand	Sandy loam
Organic matter (%)	1.5	8.0
Cation exchange capacity (meq/100g)	4.7	15.8
pH	7.6	6.7

(Determined by A&L Great Lakes Laboratories, Inc. using the Recommended Chemical Soil Test Procedures for the North Central Region, NCR No. 221)

Supplementary Table 2.2 Precursor, product ions and optimized MS/MS parameters used to quantify CBZ.

Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	DP[a](V)	EP[b] (V)	CE[d] (V)	CXP[e] (V)
CBZ	237.1	194.3	50	3.0	23.0	18.0
CBZ-d10	247.0	204.0	50	3.0	23.0	18.0

[a]declustering potential, [b]entrance potential, [c]collision energy, [d]collision cell exit potential

Supplementary Table 2.3 T-test results (*p*-values) comparing % CBZ remaining between days 4 and 14 in live microcosms and abiotic controls.

	***p*-values**			
Concentration (ng/g)	**Aerobic microcosms**	**Anaerobic microcosms**	**Aerobic abiotic controls**	**Anaerobic abiotic controls**
Soil 1. Comparison of % CBZ remaining between days 4 and 14				
50	0.0012	0.0089	NS	NS
500	0.0018	NS	*	NS
5000	0.0082	NS	NS	NS
Combined data	0.00002	0.0261	NS	NS
Soil 2. Comparison of % CBZ remaining between days 4 and 14				
50	NS	NS	Not tested	
500	NS	NS		
5000	0.0314	NS		
Combined data	NS	NS		

NS: Not significant ($p>0.05$)

*note: aerobic controls for soil 4 500 ng g^{-1} were lost during the analysis

Supplementary Table 2.4 Summary Illumina sequencing data analyzed with Mothur.

	Number of sequences following make contigs	Final number of unique sequences	Final number of sequences
Soil 1, Day 4, Aerobic	1560206	105905	994367
Soil 1, Day 14, Aerobic	1659012	112678	1065697
Soil 1, Day 4, Anaerobic	1646644	102532	1040183
Soil 1, Day 14, Anaerobic	1074021	68416	688735
Soil 2, Day 4, Aerobic	1660961	127810	1014132
Soil 2, Day 14, Aerobic	1511011	128381	928386
Soil 2, Day 4, Anaerobic	1344831	106889	804479
Soil 2, Day 14, Anaerobic	968965	82538	587506

Supplementary Table 2.5 Choa 1 average and standard deviation values (p value shown in parenthesis).

Aerobic	**Control**	**50 ng/g CBZ**	**500 ng/g CBZ**	**5000 ng/g CBZ**
Soil 1, Day 4	6724 ± 661	6056 ± 878	6832 ± 522	6576 ± 319
Soil 1, Day 14	7162 ± 547	7188± 175	6159 ± 307 **(0.050)***	6920 ± 423
Soil 2, Day 4	8216 ± 171	8425 ± 153	7583 ± 389 **(0.03)***	7900 ± 271
Soil 2, Day 14	8796 ± 725	8502 ± 438	7860 ± 571	8134 ± 1320
Anaerobic	**Control**	**50 ng/g CBZ**	**500 ng/g CBZ**	**5000 ng/g CBZ**
Soil 1, Day 4	4795 ± 1815	6678± 209	5987 ± 422	6609 ± 313
Soil 1, Day 14	4490 ± 633	4090 ± 391	6182 ± 313	5320 ± 472
Soil 2, Day 4	7355 ± 1076	6986 ± 361	6841 ± 440	7031 ± 628
Soil 2, Day 14	7018 ± 630	7180 ± 136	7165 ± 287	7069 ± 282

* a significant decrease (two tailed t-test, unequal variance) compared to the controls

Supplementary Table 2.6 Shannon diversity average and standard deviation values (p value shown in parenthesis).

Aerobic	**Control**	**50 ng/g CBZ**	**500 ng/g CBZ**	**5000 ng/g CBZ**
Soil 1, Day 4	6.82 ± 0.06	6.85± 0.08	6.92± 0	6.82 ± 0.06
Soil 1, Day 14	7.11 ± 0.02	7.0 ± 0.02 **(0.0042)***	6.89 ± 0.08 **(0.0009)***	6.93 ± 0.05 **(0.00033)***
Soil 2, Day 4	7.16 ± 0.03	7.07 ± 0.25	7.19 ± 0.01	7.19 ± 0.02
Soil 2, Day 14	7.34 ± 0.03	7.34 ± 0.02	7.31 ± 0.02	7.30 ± 0.06
Anaerobic	**Control**	**50 ng/g CBZ**	**500 ng/g CBZ**	**5000 ng/g CBZ**
Soil 1, Day 4	6.47 ± 0.23	6.76 ± 0.08	6.56 ± 0.18	6.60 ± 0.10
Soil 1, Day 14	6.33 ± 0.03	6.40 ± 0.11	6.68± 0.02 **(0.0056)**$^{+}$	6.51 ± 0.04 **(0.0386)**$^{+}$
Soil 2, Day 4	6.84 ± 0.25	6.86 ± 0.11	6.63 ± 0.11	6.68 ± 0.07
Soil 2, Day 14	7.07 ± 0.02	7.05 ± 0.02	7.03 ± 0.05	6.89 ± 0.27

+ a significant increase (two tailed t-test, unequal variance) compared to the controls
* a significant decrease (two tailed t-test, unequal variance) compared to the controls

Supplementary Table 2.7 The three most dominant OTUs in soils 1 and 2 associated with each KEGG entry as part of the drug metabolism (other enzymes) pathway. Values are gene counts contributed by each OTU and entries in bold account for the OTUs with higher gene counts.

	Day 4		Day 14		Operational Taxonomic Unit
KEGG Entry	**Average**	**Stdev**	**Average**	**Stdev**	
Thymidine phosphorylase (K00758)					
Soil 1	749	87	601	80	*Proteobacteria, Betaproteobacteria, unclassified*
	689	110	640	112	*Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Arenimonas, oryziterrae*
	258	45	312	10	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Rhodoplanes, unclassified*
Soil 2	505	102	453	69	*Proteobacteria, Betaproteobacteria, unclassified*
	325	42	325	76	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Rhodoplanes, unclassified*
	199	24	175	23	*Proteobacteria, Gammaproteobacteria, Thiotrichales, Piscirickettsiaceae, unclassified*
Uridine phosphorylase (K00757)					
Soil 1	**1232**	**170**	**1329**	**202**	***Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified***
	1262	**147**	**1101**	**205**	***Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified***
	735	221	333	114	*Verrucomicrobia, Opitutae, Opitutales, Opitutaceae, Opitutus*
Soil 2	768	91	567	47	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	532	60	487	106	*Planctomycetes, Phycisphaerae, WD2101, unclassified*
	340	19	340	127	*Verrucomicrobia, Opitutae, Opitutales, Opitutaceae, Opitutus*
Carboxylesterase 1 (K01044)					
Soil 1	219	45	222	57	*Proteobacteria, Alphaproteobacteria, BD7-3, unclassified*
	165	32	100	20	*Proteobacteria, Betaproteobacteria, MND1, unclassified*
	127	32	110	17	*Proteobacteria, Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae, unclassified*
Soil 2	106	21	271	64	*Proteobacteria, Alphaproteobacteria, BD7-3, unclassified*
	200	33	202	35	*Proteobacteria, Betaproteobacteria, MND1, unclassified*
	114	17	144	31	*Proteobacteria, Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae, unclassified*
Uridine kinase (K00876)					
Soil 1	**2062**	**225**	**2327**	**472**	***Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified***
	1262	**147**	**1101**	**205**	***Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified***
	895	189	933	215	*Acidobacteria, Acidobacteria-6, iii1-15, unclassified*
Soil 2	1492	222	1456	184	*Acidobacteria, Acidobacteria-6, iii1-15, unclassified*
	1063	87	813	96	*Verrucomicrobia, Pedosphaerae, Pedosphaerales, Ellin515, unclassified*
	712	92	639	47	*Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae, Candidatus Solibacter, unclassified*
Thymidine kinase (K00857)					
Soil 1	**2062**	**225**	**2327**	**472**	***Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified***
	1232	**170**	**1329**	**202**	***Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified***
	1232	**147**	**1101**	**205**	***Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified***
Soil 2	768	91	567	47	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	473	69	328	19	*Acidobacteria, Acidobacteria-5, unclassified*
	367	40	276	43	*Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Kaistobacter, unclassified*
Dihydropyrimidinase (K01464)					
Soil 1	406	114	509	147	*Chloroflexi, Anaerolineae, SBR1031, A4b, unclassified*
	283	146	341	159	*Actinobacteria, Actinobacteria, Actinomycetales, unclassified*
	141	46	350	14	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Xanthobacteraceae, Ancylobacter, abiegnus*
Soil 2	549	118	664	176	*Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae, unclassified*
	292	65	428	69	*Actinobacteria, Thermoleophilia, Solirubrobacterales, unclassified*
	325	42	325	76	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Rhodoplanes, unclassified*

Supplementary Table 2.7 (continued)

KEGG Entry	Day 4 Average	Day 4 Stdev	Day 14 Average	Day 14 Stdev	Operational Taxonomic Unit
Hypoxanthine phosphoribosyltransferase (K00760)					
Soil 1	**2062**	**225**	**2327**	**472**	***Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified***
	1213	**244**	**1728**	**157**	***Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae, Candidatus Solibacter, unclassified***
	1232	**170**	**1329**	**202**	***Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified***
Soil 2	1492	222	1456	184	*Acidobacteria, Acidobacteria-6, iii1-15, unclassified*
	1423	183	1277	93	*Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae, Candidatus Solibacter, unclassified*
	1063	87	813	96	*Verrucomicrobia, Pedosphaerae, Pedosphaerales, Ellin515, unclassified*
Cytidine deaminase (K01489)	**2062**	**225**	**2327**	**472**	***Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified***
Soil 1	**1235**	**170**	**1329**	**202**	***Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified***
	1259	**147**	**1101**	**205**	***Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified***
	1492	222	1456	184	*Acidobacteria, Acidobacteria-6, iii1-15, unclassified*
Soil 2	1063	87	813	96	*Verrucomicrobia, Pedosphaerae, Pedosphaerales, Ellin515, unclassified*
	655	114	659	76	*Proteobacteria, Deltaproteobacteria, Syntrophobacterales, Syntrophobacteraceae, unclassified*
Beta-glucuronidase (K01195)					
Soil 1	**2062**	**225**	**2327**	**472**	***Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified***
	1232	**170**	**1329**	**202**	***Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified***
	607	122	864	79	*Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae, Candidatus Solibacter, unclassified*
Soil 2	1063	87	813	96	*Verrucomicrobia, Pedosphaerae, Pedosphaerales, Ellin515, unclassified*
	768	91	639	47	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	712	92	567	47	*Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae, Candidatus Solibacter, unclassified*
Xanthine dehydrogenase/oxidase (K00106)					
Soil 1	236	80	146	23	*Bacteroidetes, Cytophagia, Cytophagales, Cytophagaceae, Sporocytophaga, unclassified*
	106	23	158	35	*Planctomycetes, Planctomycetia, Gemmatales, Gemmataceae, unclassified*
	109	17	109	24	*Planctomycetes, Planctomycetia, Gemmatales, Gemmataceae, Gemmata, unclassified*
Soil 2	128	10	125	9	*Planctomycetes, Planctomycetia, Gemmatales, Gemmataceae, unclassified*
	114	17	80	10	*Bacteroidetes, Cytophagia, Cytophagales, Cytophagaceae, Sporocytophaga, unclassified*
	73	22	67	8	*Planctomycetes, Planctomycetia, Gemmatales, Gemmataceae, Gemmata, unclassified*
GMP synthase (glutamine-hydrolysing) (K01951)					
Soil 1	**2062**	**225**	**2327**	**472**	***Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified***
	1213	**198**	**1546**	**384**	***Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Kaistobacter, unclass.***
	1378	**221**	**1280**	**224**	***Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Arenimonas, oryziterrae***
Soil 2	1492	222	1327	352	*Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae, unclassified*
	1310	228	1456	184	*Acidobacteria, Acidobacteria-6, iii1-15, unclassified*
	1098	228	1318	152	*Proteobacteria, Deltaproteobacteria, Syntrophobacterales, Syntrophobacteraceae, unclassified*
Arylamine N-acetyltransferase (K00622)					
Soil 1	80	18	106	11	*Proteobacteria, Alphaproteobacteria, unclassified*
	56	21	71	24	*Actinobacteria, Actinobacteria, Actinomycetales, Mycobacteriaceae, Mycobacterium, vaccae*
	31	12	32	7	*Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Novosphingobium, unclassified*
Soil 2	44	11	54	27	*Proteobacteria, Alphaproteobacteria, unclassified*
	14	3	21	4	*Actinobacteria, Actinobacteria, Actinomycetales, Mycobacteriaceae, Mycobacterium, vaccae*
	12	1	11	2	*Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Novosphingobium, unclassified*

Supplementary Table 2.7 (continued)

KEGG Entry	Day 4 Average	Day 4 Stdev	Day 14 Average	Day 14 Stdev	Operational Taxonomic Unit
IMP dehydrogenase (K00088)					
Soil 1	**2062**	**225**	**2327**	**472**	***Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified***
	1232	**170**	**1329**	**202**	***Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified***
	1262	**147**	**1101**	**205**	***Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified***
Soil 2	1492	222	1456	184	*Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae, unclassified*
	1098	236	1327	352	*Acidobacteria, Acidobacteria-6, iii1-15, unclassified*
	1048	113	813	96	*Verrucomicrobia, Pedosphaerae, Pedosphaerales, Ellin515, unclassified*
Dihydropyrimidine dehydrogenase (K00207)					
Soil 1	127	32	110	17	*Proteobacteria, Alphaproteobacteri, Rhodospirillales, Rhodospirillaceae, unclassified*
	112	17	47	19	*Proteobacteria, Betaproteobacteria, Burkholderiales, Oxalobacteraceae, Massilia, unclassified*
	80	18	106	54	*Proteobacteria, Alphaproteobacteria, unclassified*
Soil 2	114	17	144	31	*Proteobacteria, Alphaproteobacteri, Rhodospirillales, Rhodospirillaceae, unclassified*
	44	11	54	4	*Proteobacteria, Alphaproteobacteria, unclassified*
	19	6	19	5	*Proteobacteria, Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae, Reyranella, massiliensis*
Uridine monophosphate synthetase (K13421)					
Soil 1	**1262**	**147**	**1101**	**205**	***Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified***
	406	114	509	147	*Chloroflexi, Anaerolineae, SBR1031, A4b, unclassified*
	38	11	65	18	*Chloroflexi, Anaerolineae, SBR1031, SJA-101, unclassified*
Soil 2	290	28	208	38	*Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified*
	168	32	212	23	*Proteobacteria, Alphaproteobacteria, unclassified*
	160	36	206	13	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Devosia, unclassified*
Thiopurine S-methyltransferase (K00569)					
Soil 1	**1262**	**147**	**826**	**205**	***Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified***
	689	110	640	112	*Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Arenimonas, oryziterrae*
	466	84	462	76	*Gemmatimonadetes, Gemm-1, unclassified*
Soil 2	440	82	403	72	*Gemmatimonadetes, Gemm-1, unclassified*
	288	72	279	49	*WS3, PRR-12, Sediment-1, PRR-10, unclassified*
	219	30	275	41	*Proteobacteria, Deltaproteobacteria, Myxococcales, Haliangiaceae, unclassified*

Supplementary Table 2. 8 The three most dominant OTUs in soils 1 and 2 associated with each KEGG entry as part of the drug metabolism (cytochrome P450) pathway.Values are gene counts contributed by each OTU and entries in bold account for the OTUs with higher gene counts.

	Day 4		Day 14		Operational Taxonomic Unit
KEGG Entry	**Average**	**Stdev**	**Average**	**Stdev**	
Monoamine oxidase (K00274)					
Soil 1	**2464**	**340**	**2658**	**403**	***Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified***
	2062	**225**	**2327**	**472**	***Acidobacteria, Chloracidobacteria, RB41, Ellin6075, unclassified***
	1262	**147**	**1101**	**205**	***Bacteroidetes, Saprospirae, Saprospirales, Saprospiraceae, unclassified***
Soil 2	1536	182	1134	94	*Bacteroidetes, Saprospirae, Saprospirales, Chitinophagaceae, unclassified*
	817	90	639	47	*Verrucomicrobia, Spartobacteria, Chthoniobacterales, Chthoniobacteraceae, DA101, unclassified*
	712	92	639	55	*Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae, Candidatus Solibacter, unclassified*
Dimethylaniline monooxygenase (K00485)					
Soil 1	258	45	312	10	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Rhodoplanes, unclassified*
	219	45	222	57	*Proteobacteria, Alphaproteobacteria, BD7-3, unclassified*
	89	21	133	28	*Proteobacteria, Deltaproteobacteria, Myxococcales, Haliangiaceae, unclassified*
Soil 2	325	42	325	76	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Rhodoplanes, unclassified*
	30	30	271	64	*Proteobacteria, Deltaproteobacteria, Myxococcales, Haliangiaceae, unclassified*
	106	21	275	49	*Proteobacteria, Alphaproteobacteria, BD7-3, unclassified*
Aldehyde dehydrogenase (K00129)					
Soil 1	109	19	137	36	*Planctomycetes, Planctomycetia, Planctomycetales, Planctomycetaceae, Planctomyces, unclassified*
	67	22	108	28	*Proteobacteria, Deltaproteobacteria, Myxococcales, Cystobacteraceae, Cystobacter, fuscus*
	56	21	73	29	*Actinobacteria, Actinobacteria, Actinomycetales, Intrasporangiaceae, unclassified*
Soil 2	132	30	171	24	*Proteobacteria, Deltaproteobacteria, Myxococcales, Cystobacteraceae, Cystobacter, fuscus*
	120	12	107	27	*Proteobacteria, Deltaproteobacteria, Myxococcales, Myxococcaceae, Anaeromyxobacter, unclassified*
	96	4	126	3	*Proteobacteria, Deltaproteobacteria, Myxococcales, Cystobacteraceae, Angiococcus, disciformis*
Glutathione S-transferase (K00799)					
Soil 1	**5457**	**891**	**6958**	**1727**	***Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Kaistobacter, unclass.***
	5457	**901**	**6242**	**198**	***Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Rhodoplanes, unclassified***
	4135	663	3128	603	*Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Arenimonas, oryziterrae*
Soil 2	6502	835	6505	1524	*Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Rhodoplanes, unclassified*
	3302	360	2484	384	*Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Kaistobacter, unclass.*
	2190	300	2753	384	*Proteobacteria, Deltaproteobacteria, Myxococcales, Haliangiaceae, unclassified*

REFERENCES

REFERENCES

Acosta-Gonzalez A, Martirani-von Abercron SM, Rossello-Mora R, Wittich RM, Marques S. 2015. The effect of oil spills on the bacterial diversity and catabolic function in coastal sediments: a case study on the Prestige oil spill. Environmental Science and Pollution Research. 22, 15200-15214.

Al-Mailem DM, Kansour MK, Radwan SS. 2014. Bioremediation of hydrocarbons contaminating sewage effluent using man-made biofilms: effects of some variables. Applied Biochemistry and Biotechnology.174, 1736-1751.

Ardley JK, Parker MA, De Meyer SE, Trengove RD, O'Hara GW, Reeve WG, et al. 2012. Microvirga lupini sp nov., *Microvirga lotononidis* sp nov and *Microvirga zambiensis* sp nov are alphaproteobacterial root-nodule bacteria that specifically nodulate and fix nitrogen with geographically and taxonomically separate legume hosts. International Journal of Systematic and Evolutionary Microbiology. 62, 2579-2588.

Arye G, Dror I, Berkowitz B. 2011. Fate and transport of carbamazepine in soil aquifer treatment (SAT) infiltration basin soils. Chemosphere. 82, 244-252.

Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG, Walters WA, et al. 2011. The under-recognized dominance of *Verrucomicrobia* in soil bacterial communities. Soil Biology & Biochemistry. 43, 1450-1455.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME Journal. 6, 1621-1624.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al.2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America. 108, 4516-4522.

Cha JM, Cupples AM. 2009. Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids. Water Research. 43, 2522-2530.

Chen F, Ying GG, Kong LX, Wang L, Zhao JL, Zhou LJ, et al. 2011. Distribution and accumulation of endocrine-disrupting chemicals and pharmaceuticals in wastewater irrigated soils in Hebei, China. Environmental Pollution. 159, 1490-1498.

Chen K, Chen Q, Wang GX, Ni HY, He J, Yan X, et al. 2015. *Sphingomonas chloroacetimidivorans* sp. nov., a chloroacetamide herbicide-degrading bacterium isolated from activated sludge. Antonie Van Leeuwenhoek. 108, 703-10.

Clara M, Strenn B, Kreuzinger N. 2004. Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration. Water Research. 38, 947-954.

Conkle JL, Gan J, Anderson MA. 2012. Degradation and sorption of commonly detected PPCPs in wetland sediments under aerobic and anaerobic conditions. Journal of Soils and Sediments. 12, 1164-1173.

Coronado E, Valtat A, van der Meer JR.2015. *Sphingomonas wittichii* RW1 gene reporters interrogating the dibenzofuran metabolic network highlight conditions for early successful development in contaminated microcosms. Environmental Microbiology Reports.7, 480-8.

Dalkmann P, Siebe C, Amelung W, Schloter M, Siemens J. 2014. Does long-term irrigation with untreated wastewater accelerate the dissipation of pharmaceuticals in soil? Environmental Science & Technology. 48, 4963-4970.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology. 72, 5069-5072.

Ding GC, Heuer H, Smalla K. 2012. Dynamics of bacterial communities in two unpolluted soils after spiking with phenanthrene: soil type specific and common responders. Frontiers in Microbiology. 3, 290.

Duran-Alvarez JC, Prado B, Gonzalez D, Sanchez Y, Jimenez-Cisneros B. 2015. Environmental fate of naproxen, carbamazepine and triclosan in wastewater, surface water and wastewater irrigated soil - Results of laboratory scale experiments. Science of the Total Environment. 538, 350-362.

Gauthier H, Yargeau V, Cooper DG. 2010. Biodegradation of pharmaceuticals by *Rhodococcus rhodochrous* and *Aspergillus niger* by co-metabolism. Science of the Total Environment. 408, 1701-1706.

Gibson R, Duran-Alvarez JC, Estrada KL, Chavez A, Cisneros BJ. 2010. Accumulation and leaching potential of some pharmaceuticals and potential endocrine disruptors in soils irrigated with wastewater in the Tula Valley, Mexico. Chemosphere. 81, 1437-1445.

Gottschall N, Topp E, Metcalfe C, Edwards M, Payne M, Kleywegt S, et al. 2012. Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field. Chemosphere. 87, 194-203.

Grossberger A, Hadar Y, Borch T, Chefetz B. 2014. Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. Environmental Pollution. 185, 168-177.

Gutierrez T, Singleton DR, Aitken MD, Semple KT. 2011. Stable isotope probing of an algal bloom to identify uncultivated members of the Rhodobacteraceae associated with low-molecular-weight polycyclic aromatic hydrocarbon degradation. Applied and Environmental Microbiology. 77, 7856-60.

Harwati TU, Kasai Y, Kodama Y, Susilaningsih D, Watanabe K. 2009. *Tropicibacter naphthalenivorans* gen. nov., sp nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from Semarang Port in Indonesia. International Journal of Systematic and Evolutionary Microbiology. 59, 392-396.

Janssen PH. 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and Environmental Microbiology. 72, 1719-1728.

Janssen PH, Schuhmann A, Morschel E, Rainey FA. 1997. Novel anaerobic ultramicrobacteria belonging to the *Verrucomicrobiales* lineage of bacterial descent isolated by dilution culture from anoxic rice paddy soil. Applied and Environmental Microbiology. 63, 1382-1388.

Joseph SJ, Hugenholtz P, Sangwan P, Osborne CA, Janssen PH. 2003. Laboratory cultivation of widespread and previously uncultured soil bacteria. Applied and Environmental Microbiology. 69, 7210-7215.

Kanso S, Patel BKC. 2003. *Microvirga subterranea* gen. nov., sp nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. International Journal of Systematic and Evolutionary Microbiology 53, 401-406.

Kinney CA, Furlong ET, Werner SL, Cahill JD. 2006a. Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. Environmental Toxicology and Chemistry. 25, 317-326.

Kinney CA, Furlong ET, Zaugg SD, Burkhardt MR, Werner SL, Cahill JD, et al. 2006b. Survey of organic wastewater contaminants in biosolids destined for land application. Environmental Science & Technology. 40, 7207-7215.

Kobayashi A, Kobayashi YO, Someya N, Ikeda S. 2015. Community analysis of root- and tuber-associated bacteria in field-grown potato plants harboring different resistance levels against common scab. Microbes and Environments. 30, 301-309.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental Science & Technology. 36, 1202-1211.

Lajeunesse A, Smyth SA, Barclay K, Sauve S, Gagnon C. 2012. Distribution of antidepressant residues in wastewater and biosolids following different treatment processes by municipal wastewater treatment plants in Canada. Water Research. 46, 5600-5612.

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology. 31, 814-821.

Lapworth DJ, Baran N, Stuart ME, Ward RS. 2012. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. Environmental Pollution.163, 287-303.

Li JY, Dodgen L, Ye QF, Gan J.2013. Degradation kinetics and metabolites of carbamazepine in soil. Environmental Science & Technology. 47, 3678-3684.

Maeng SK, Sharma SK, Abel CDT, Magic-Knezev A, Amy GL. 2011. Role of biodegradation in the removal of pharmaceutically active compounds with different bulk organic matter characteristics through managed aquifer recharge: Batch and column studies. Water Research. 45, 4722-4736.

Matsumura Y, Akahira-Moriya A, Sasaki-Mori M. 2015. Bioremediation of bisphenol-A polluted soil by *Sphingomonas bisphenolicum* AO1 and the microbial community existing in the soil. Biocontrol Sciences. 20, 35-42.

McClellan K, Halden RU. 2010. Pharmaceuticals and personal care products in archived US biosolids from the 2001 EPA national sewage sludge survey. Water Research. 44, 658-668.

Metcalfe CD, Koenig BG, Bennie DT, Servos M, Ternes TA, Hirsch R.2003. Occurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants. Environmental Toxicology and Chemistry. 22, 2872-2880.

Miao XS, Metcalfe CD. 2003. Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography-electrospray tandem mass spectrometry. Analytical Chemistry. 75, 3731-3738.

Miao XS, Yang JJ, Metcalfe CD. 2005. Carbamazepine and its metabolites in wastewater and in biosolids in a municipal wastewater treatment plant. Environmental Science & Technology. 39, 7469-7475.

Miege C, Choubert JM, Ribeiro L, Eusebe M, Coquery M. 2008. Removal efficiency of pharmaceuticals and personal care products with varying wastewater treatment processes and operating conditions - conception of a database and first results. Water Science and Technology. 57, 49-56.

Nadh AG, Akare UR, Sharma P, Yadav P, Bandaru S, Nayarisseri A, et al. 2015. Identification of azo dye degrading *Sphingomonas* strain EMBS022 and EMBS023 using 16S rRNA gene sequencing. Current Bioinformatics. 10, 599-605.

Nakada N, Kiri K, Shinohara H, Harada A, Kuroda K, Takizawa S, et al. 2008. Evaluation of pharmaceuticals and personal care products as water-soluble molecular markers of sewage. Environmental Science & Technology. 42, 6347-6353.

Nayarisseri A, Suppahia A, Nadh AG, Nair AS. 2015. Identification and characterization of a pesticide degrading *Flavobacterium* species EMBS0145 by 16S rRNA gene sequencing. Interdisciplinary Sciences-Computational Life Sciences. 7, 93-99.

Ollers S, Singer HP, Fassler P, Muller SR. 2001. Simultaneous quantification of neutral and acidic pharmaceuticals and pesticides at the low-ng/1 level in surface and waste water. Journal of Chromatography A. 911, 225-234.

Pan T, Deng T, Zeng XY, Dong W, Yu SJ. 2016. Extractive biodegradation and bioavailability assessment of phenanthrene in the cloud point system by *Sphingomonas polyaromaticivorans*. Applied Microbiology and Biotechnology. 100, 431-437.

Perruchon C, Patsioura V, Vasileiadis S, Karpouzas DG. 2016. Isolation and characterisation of a *Sphingomonas* strain able to degrade the fungicide ortho-phenylphenol. Pest Management Science. 72, 113-124.

Pinyakong O, Tiangda K, Iwata K, Omori T. 2012. Isolation of novel phenanthrene-degrading bacteria from seawater and the influence of its physical factors on the degradation of phenanthrene. Scienceasia. 38, 36-43.

Popa C, Favier L, Dinica R, Semrany S, Djelal H, Amrane A, et al. 2014. Potential of newly isolated wild *Streptomyces* strains as agents for the biodegradation of a recalcitrant pharmaceutical, carbamazepine. Environmental Technology. 35, 3082-3091.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, et al. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Research. 35, 7188-7196.

Radl V, Simoes-Araujo JL, Leite J, Passos SR, Martins LMV, Xavier GR, et al. 2014. *Microvirga vignae* sp nov., a root nodule symbiotic bacterium isolated from cowpea grown in semi-arid Brazil. International Journal of Systematic and Evolutionary Microbiology. 64, 725-730.

Reeve W, Ardley J, Tian R, De Meyer S, Terpolilli J, Melino V, et al. 2014. Genome sequence of the *Listia angolensis* microsymbiont *Microvirga lotononidis* strain WSM3557(T). Standards in Genomic Sciences. 9, 540-550.

Rosenberg E. 2014. The Family *Chitinophagaceae*. In: E. R, F. DE, S. L, E. S, F. T, editors. The Prokaryotes - Other Major Lineages of Bacteria and Archaea. Springer-Verlag. pp.493-495.

Rosi-Marshall EJ, Royer TV. 2012. Pharmaceutical compounds and ecosystem function: an emerging research challenge for aquatic ecologists. Ecosystems. 15, 867-880.

Salvia MV, Vulliet E, Wiest L, Baudot R, Cren-Olive C. 2012. Development of a multi-residue method using acetonitrile-based extraction followed by liquid chromatography-tandem mass spectrometry for the analysis of steroids and veterinary and human drugs at trace levels in soil. Journal of Chromatography A. 1245, 122-133.

Santos P, Pinhal I, Rainey FA, Empadinhas N, Costa J, Fields B, et al. 2003. Gamma-proteobacteria *Aquicella lusitana* gen. nov., sp nov., and *Aquicella siphonis* sp nov infect protozoa and require activated charcoal for growth in laboratory media. Applied and Environmental Microbiology. 69, 6533-6540.

Schloss PD. 2009. A High-Throughput DNA Sequence Aligner for Microbial Ecology Studies. Plos One. 4.

Schloss PD. 2013. MiSeq SOP. In: Schloss PD, editor. Mothur wiki. 2013, pp. This is a page describing the standard operating procedure for analysing MiSeq data using Mothur.

Schmitt H, Van Beelen P, Tolls J, Van Leeuwen CL. 2004. Pollution-induced community tolerance of soil microbial communities caused by the antibiotic sulfachloropyridazine. Environmental Science & Technology. 38, 1148-1153.

Syakti AD, Yani M, Hidayati NV, Siregar AS, Doumenq P, Sudiana IMM. 2013. The bioremediation potential of hydrocarbonoclastic bacteria isolated From a mangrove contaminated by petroleum hydrocarbons on the Cilacap Coast, Indonesia. Bioremediation Journal. 17, 11-20.

Ternes TA. 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Research. 32, 3245-3260.

Thiele-Bruhn S. 2003. Pharmaceutical antibiotic compounds in soils - a review. Journal of Plant Nutrition and Soil Science. 166, 145-167.

Thiele-Bruhn S, Beck IC. 2005. Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. Chemosphere. 59, 457-465.

Tixier C, Singer HP, Oellers S, Muller SR. 2003. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environmental Science & Technology. 37, 1061-1068.

Tran NH, Li JH, Hu JY, Ong SL. 2014. Occurrence and suitability of pharmaceuticals and personal care products as molecular markers for raw wastewater contamination in surface water and groundwater. Environmental Science and Pollution Research. 21, 4727-4740.

Vazquez-Roig P, Segarra R, Blasco C, Andreu V, Pico Y. 2010. Determination of pharmaceuticals in soils and sediments by pressurized liquid extraction and liquid chromatography tandem mass spectrometry. Journal of Chromatography A. 1217, 2471-2483.

Vieno N, Sillanpaa M. 2014. Fate of diclofenac in municipal wastewater treatment plant - A review. Environment International. 69, 28-39.

Walters E, McClellan K, Halden RU. 2010. Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. Water Research. 44, 6011-6020.

Williams CF, McLain JET. 2012. Soil persistence and fate of carbamazepine, lincomycin, caffeine, and ibuprofen from wastewater reuse. Journal of Environmental Quality. 41, 1473-1480.

Wu CX, Spongberg AL, Witter JD, Fang M, Czajkowski KP, Ames A. 2010. Dissipation and leaching potential of selected pharmaceutically active compounds in soils amended with biosolids. Archives of Environmental Contamination and Toxicology. 59, 343-351.

Xia F, Zou B, Shen C, Zhu T, Gao XH, Quan ZX. 2015. Complete genome sequence of *Methylophilus* sp TWE2 isolated from methane oxidation enrichment culture of tap-water. Journal of Biotechnology. 211, 121-122.

Yergeau E, Sanschagrin S, Waiser MJ, Lawrence JR, Greer CW. 2012. Sub-inhibitory concentrations of different pharmaceutical products affect the meta-transcriptome of river biofilm communities cultivated in rotating annular reactors. Environmental Microbiology Reports. 4, 350-359.

Yomantas YAV, Tokmakova IL, Gorshkova NV, Abalakina EG, Kazakova SM, Gak ER, et al. 2010. Aromatic amino acid auxotrophs constructed by recombinant marker exchange in *Methylophilus methylotrophu*s AS1 cells expressing the *aroP*-encoded transporter of *Escherichia coli*. Applied and Environmental Microbiology.76, 75-83.

Zacharoff L, Chan CH, Bond DR. 2016. Reduction of low potential electron acceptors requires the CbcL inner membrane cytochrome of Geobacter sulfurreducens. Bioelectrochemistry. 107, 7-13.

Chapter 3:

Diclofenac, Carbamazepine and Triclocarban Biodegradation in Agricultural Soils and the Microorganisms and Metabolic Pathways Affected

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3.1. Abstract

The incomplete elimination of pharmaceuticals and personal care products (PPCPs) during wastewater treatment has resulted in their detection in the environment. PPCP biodegradation is a potential removal mechanism; however, the microorganisms and pathways involved in soils are generally unknown. Here, the biodegradation of diclofenac (DCF), carbamazepine (CBZ) and triclocarban (TCC) in four agricultural soils at concentrations typically detected in soils and biosolids (50 ng g^{-1}) was examined. Rapid DCF removal (<7 days) was observed under aerobic conditions, but only limited biodegradation was noted under other redox conditions. CBZ and TCC degradation under aerobic conditions was slow (half-lives of 128-241 days and 165-190 days for CBZ and TCC). Phylotypes in the *Proteobacteria, Gemmatimonadales* and *Actinobacteria* were significantly more abundant during DCF biodegradation compared to the controls (no DCF). For CBZ, those in the *Bacteroidetes, Actinobacteria, Proteobacteria* and *Verrucomicrobia* were enriched compared to the controls. *Actinobacteria* and *Proteobacteria* were also enriched during TCC biodegradation. Such differences could indicate these microorganisms are associated with the biodegradation of these compounds, as they appear to be

benefiting from their removal. The impact of these PPCPs on the KEGG pathways associated with metabolism was also examined. Four pathways were positively impacted during DCF biodegradation (propanoate, lysine, fatty acid & benzoate metabolism). These pathways are likely common in soils, explaining the rapid removal of DCF. There was limited impact of CBZ on the metabolic pathways. TCC removal was linked to genes associated with the degradation of simple and complex substrates. The results indicate even low concentrations of PPCPs significantly affect soil communities. The recalcitrant nature of TCC and CBZ suggests soils receiving biosolids could accumulate these chemicals, representing risks concerning crop uptake.

3.2. Introduction

Pharmaceuticals and personal care products (PPCPs) in the environment represent a significant problem because of the known or suspected adverse effects associated with these compounds. For instance, certain PPCPs cause microbial resistance (Gao et al., 2012; Middleton and Salierno, 2013), exhibit acute toxicity (Hussain et al., 2008) or cause endocrine disruption (Bevans et al., 1996; Harries et al., 1996). Municipal wastewater treatment plants represent a route of entry of PPCPs into the environment (Daughton and Ternes, 1999; Wu et al., 2010b). During wastewater treatment, PPCPs undergo partial removal, leading to their occurrence in wastewater effluents and biosolids (Cha and Cupples, 2009; Clara et al., 2005; Miao and Metcalfe, 2003; Wu et al., 2010b). The reuse of treated wastewater for irrigation and biosolids application are pathways for the transport of PPCPs into soils (Li et al., 2013b; Wu et al., 2010b; Ying et al., 2007). Concerns have been raised for agricultural soils because of the risk of movement into the food chain. For example, several studies have provided evidence regarding the uptake of PPCPs by crops (Dodgen et al., 2013; Holling et al., 2012; Shenker et al., 2011;

Wu et al., 2010b). To better characterize the risks associated with these emerging contaminants, there is a need to better understand the processes that govern their fate in agricultural soils.

Among common PPCPs detected in wastewater and biosolids are the non-steroidal anti-inflammatory drug diclofenac (2-(2, 6-dichloranilino) phenylacetic acid) (DCF) (Radjenovic et al., 2009b; Ternes, 2000), the anti-seizure drug carbamazepine (5H-dibenzo [b, f] azepine-5-carboxamide) (CBZ) (Miao et al., 2005; Zhang et al., 2008), and the antimicrobial agent triclocarban (3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)urea (TCC) (Lozano et al., 2013; Ogunyoku and Young, 2014). In biosolids, DCF has been detected at concentrations ranging from $\leq$10 ng g^{-1} to 627 ng g^{-1}(Albero et al., 2014; Morais et al., 2013; Radjenovic et al., 2009b; Spongberg and Witter, 2008). CBZ in biosolids has typically ranged from 5 ng g^{-1} to up to 258 ng g^{-1}(Ding et al., 2011; Gottschall et al., 2012; Morais et al., 2013; Radjenovic et al., 2009a; Sabourin et al., 2012; Spongberg and Witter, 2008). Higher concentrations of TCC have been detected in biosolids, ranging from 2,900 to 51,000 ng g^{-1}, due to its hydrophobic nature (Cha and Cupples, 2009; Chu and Metcalfe, 2007; Gottschall et al., 2012; Guerra et al., 2014; Sabourin et al., 2012; Wu et al., 2010a). These chemicals have also been detected in soils amended with biosolids or irrigated with wastewater (Cha and Cupples, 2009; Chen et al., 2011; Gibson et al., 2010; Walker et al., 2012; Walters et al., 2010). In most cases, DCF in soils was undetected (Dalkmann et al., 2012; Vazquez-Roig et al., 2011; Vazquez-Roig et al., 2010) or low (0.09 ng g^{-1}) (Azzouz and Ballesteros, 2012). CBZ in soils has ranged between 1.8 and 7.5 ng g^{-1} (Gibson et al., 2010; Vazquez-Roig et al., 2010; Walker et al., 2012) whereas TCC has ranged from 1.2 to 200 ng g^{-1}(Cha and Cupples, 2009; Chen et al., 2011; Wu et al., 2010a). The detection of PPCPs in soils and biosolids is problematic because they have the potential to be bioactive and/or potent at low doses (Daughton and Ternes, 1999). For example, DCF (100 µg

DCF l^{-1}) altered the structure of algal and bacterial communities of river biofilms and a reduction in bacterial river biomass was attributed to CBZ (10 µg CBZ l^{-1}) (Lawrence et al., 2005). In anaerobic digesters, TCC (although at a higher concentration, 6.8 x 10^5 ng g^{-1}) caused a decrease in methane production, an increase in antibiotic resistance genes, and a shift in the structure of the underlying microbial community (Carey et al., 2016). Furthermore, TCC (316–3156 µg TCC l^{-1}) caused effects associated with endocrine disruption (Ahn et al., 2008; Chen et al., 2008).

A well-recognized removal mechanism of xenobiotics in soils involves their biodegradation by soil bacteria. However, the specific bacteria and pathways involved in DCF, CBZ and TCC biodegradation in agricultural soils are generally unknown. To date, the majority of information on DCF degrading bacteria has been obtained from other microbial sources/inocula, rather than from agricultural soils and these experiments have been performed under aerobic conditions. For example, an *Actinoplanes* sp. (from a fiber cartridge reactor) (Osorio-Lozada et al., 2008), *Enterobacter hormaechei* D15 (from activated sludge) (Aissaoui et al., 2017a), *Enterobacter cloacae* (from household compost) (Aissaoui et al., 2017b) and *Brevibacterium* sp. D4 (from activated sludge) (Bessa et al., 2017), all transformed DCF. *Sphingomonas* sp. and other bacteria removed DCF in reactors inoculated with activated sludge or ditch sediment (Kim et al., 2017).

Isolates have also been associated with CBZ and TCC biodegradation. *Rhodococcus rhodochrous* (from ATCC) and activated sludge isolates *Starkeya* sp. C11, *Rhizobium* sp. C12 and *Pseudomonas* sp. CBZ-4 degraded CBZ in liquid media (Gauthier et al., 2010) (Bessa et al., 2017; Li et al., 2013a). To our knowledge, one soil isolate, *Streptomyces* MIUG 4.89, has been linked to CBZ biodegradation, also transforming CBZ in liquid media (Popa et al., 2014). As for TCC, unclassified *Alcaligenacae* were associated with TCC degradation in an enrichment culture derived from activated sludge (Miller et al., 2008). More recently, *Sphingomonas* sp. strain YL-

JM2C and *Ochrobactrum* sp. TCC-1 (both from activated sludge) degraded TCC in liquid media (Mulla et al., 2016; Yun et al., 2017a). *Ochrobactrum* sp. TCC-2 (from river sediment) degraded TCC under both aerobic and nitrate reducing conditions (Yun et al., 2017b). Further, two soil isolates, *Pseudomonas fluorescens* MC46 and *Ochrobactrum* sp. MC22, transformed TCC in pot soils and in mineral salt media (Sipahutar et al., 2018; Sipahutar and Vangnai, 2017). Although these studies clearly indicate that specific bacteria are capable of DCF, TCC and CBZ biodegradation, it is unknown if these microorganisms are capable of degrading these chemicals in agricultural soils when PPCPs are present at lower concentrations.

The current study aimed to generate microbiological data on DCF, CBZ and TCC biodegradation for conditions that are more reflective of those found in soil environments. Specifically, the experiments were conducted with agricultural soils amended with 50 ng g^{-1}. Further, biodegradation experiments were performed using soil and not in liquid media. Additionally, to our knowledge, for the first time, attempts were made to determine metabolic pathways impacted by these PCPPs, to enable the generation of hypotheses concerning removal mechanisms. The objectives were to 1) determine the susceptibility of DCF, CBZ, and TCC to biodegradation in agricultural soils at ppb concentrations, 2) identify microorganisms which increase in abundance during DCF, CBZ and TCC biodegradation and 3) determine which pathways are putatively linked to the biological removal of these chemicals. While DCF biodegradation was studied over four redox conditions (aerobic, nitrate reducing, sulfate reducing and methanogenic), CBZ and TCC removal was only examined under aerobic conditions due to the recalcitrant nature of these two chemicals.

3.3. Methods

3.3.1. Chemicals and Materials

Diclofenac sodium (purity >99%) was obtained at Santa Cruz Biotechnology (Dallas, Texas, USA). CBZ and TCC (purity >99%) were purchased from Sigma-Aldrich (Saint-Louis, MO, USA). DCF-d_4 and CBZ-d_{10} (purity >99.4%) were acquired from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada) and TCC-$^{13}C_6$ was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). HPLC grade acetonitrile and LC/MS grade methanol were purchased from Fisher Scientific and Michigan State University (MSU) Biochemistry and Molecular Biology store. QuEChERS (quick, easy, cheap, effective, rugged, and safe) extract pouches (6.0 g magnesium sulfate and 1.5 g sodium acetate) were purchased from Agilent Technologies (Wilmington, DE, USA). Medical grade talcum powder was obtained from Fisher Scientific (Dallas, TX, USA). The Cleanert SAX (1000mg, 6 cc), Oasis HLB (200 mg, 6cc), and Strata™-X (200 mg, 6 cc) cartridges were purchased from GS-Tek (Newark, DE, USA), Waters (Milford, MA, USA), and Phenomenex (Torrance, CA, USA), respectively. The DNA isolation kit (PowerSoil) was obtained at MO BIO Laboratories (Carlsbad, CA, USA).

3.3.2. Soils and the Experimental Design

Soils collected from agricultural areas within Michigan were sieved (2-mm diameter) to remove the coarse particles and were stored at 4^{o}C until use. The soil samples comprised three sandy loam soils (herein A, B, D) and one loamy sand soil (herein C). Select soil properties, determined by A & L Great Lakes Laboratories, Inc. (Fort Wayne, IN), included pH values of 6.9 for the sandy loam soils (A, B, D) and 6.6 for the loamy sand soil (C). The soil organic matter values (determined by A & L Great Lakes Laboratories) were 2.8%, 2.4%, 1.4% and 1.9% for soils A, B, C, and D, respectively and were obtained using the loss on ignition method

(North Central Regional Research Publication No. 221).

Batch soil microcosms to investigate DCF biodegradation under three terminal electron-accepting process (TEAP) conditions (nitrate-reducing, sulfate-reducing, and methanogen) were established in 30-ml sterile amber glass bottles using soils A and C (selected to represent a range in organic matter). For these, soil samples (5 g wet weight) were placed into the bottles in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) along with autoclaved DI water and filtered stock solutions of sodium nitrate and sodium sulfate. The next day, for each redox condition, nine live microcosms (3 time points x triplicates, sacrificial experimental design) and nine abiotic controls (obtained by autoclaving for 45 min on three consecutive days) were prepared by adding 10 ml of DI water into the serum bottles containing the soil. To establish nitrate-reducing conditions, sodium nitrate (5 mM) was added to the microcosms and their abiotic controls. Nitrate concentrations were measured with ion chromatography (Dionex series 2000i/SP, 4 x 250 mm; Dionex IonPac® AS4A-SC Sunnyvale, CA) 13 days later. After a decrease (>60%) in nitrate (another 13 days later), the soil microcosms were spiked with 500 µl of 500 µg l^{-1} DCF in DI water and sodium nitrate was again added. Sulfate-reducing conditions were established in similar fashion with sodium sulfate, except that 500 µl of 500 µg l^{-1} DCF was added to the microcosms after 28 days. No further additions of sodium nitrate or sodium sulfate were needed for the abiotic microcosms upon spiking with DCF since no decrease was observed. For the methanogenic conditions, methane was measured twice (after 3 weeks and 45 days) using gas chromatography (PerkinElmer, Waltham, MA) as previously described (except the oven temperature was set to 40^{o}C, elution time, 1 minute) (Jayamani and Cupples, 2015). Upon methane detection, the microcosms were spiked with 50 ng DCF g^{-1} (after 46 days). All microcosms were crimp-sealed with rubber stoppers following the addition of DCF. At three

time points (days 7, 21, and 50), DCF was sacrificially extracted from each sample microcosm and abiotic control.

Another three batch soil experiments were performed to study DCF, CBZ or TCC biodegradation under aerobic conditions using all four soils. For each PPCP, the experimental design again involved sacrificial sampling and included nine live microcosms (3 time points x triplicates), nine live controls (treated in the same manner as the live microcosms except no PPCP added) and nine abiotic controls. Here, soil samples (5 g, wet weight) were placed into sterile serum bottles (30 ml) and these were spiked with 500 µl of 500 µg l^{-1} filtered DCF or CBZ working solution in DI water to reach a concentration of 50 ng g^{-1}. After vortex mixing, the water content of the soil was adjusted to 60% of the soil maximum water holding capacity by adding sterilized DI water. TCC was introduced into the microcosms using talcum powder as a carrier due to the poor water solubility of TCC. Talcum powder was used to avoid introducing organic solvent into the microcosms, which can serve as carbon source or potentially inhibit biodegradation (Dasu et al., 2013; Khan and Lee, 2010). Briefly, 4 g of talcum powder was spiked with 2 ml of 10 mg l^{-1} TCC working solution in acetonitrile (Khan and Lee, 2010). The content was mixed with a sterile spatula and left under a fume hood until complete evaporation of the solvent. Then, 50 mg of the talc-coated TCC was thoroughly mixed with 5 g of soil to obtain a concentration of 50 ng TCC g^{-1} soil. The moisture content of the soil was adjusted as described above. Talcum powder was equally added to the live controls, except that acetonitrile was not fortified with TCC. The serum bottles were partially capped with septa and incubated in dark cabinets. Water content of the soils was regularly monitored by weighing the bottles, and sterilized DI water was added to offset any water loss. Following incubation, the chemicals were extracted from the sample microcosms and abiotic controls on days 3 and 7 (DCF), days 4, 21,

and 40 (CBZ) and days 4, 21 and 50 (TCC). These time points were selected based on previous studies illustrating the rapid removal of DCF compared to CBZ and TCC. The TCC degradation experiment was repeated over a longer period of time (70 days) employing a similar experimental design as described above.

The first order exponential decay model ($C_t = C_o e^{-kt}$) was used to fit CBZ and TCC concentration data to estimate half-life values ($t_{1/2}$) using the degradation rate constant k (d^{-1}). Here, C_t and C_o represent CBZ and TCC concentrations remaining in soils (ng g^{-1}) after time t (days) and the initial concentrations (ng g^{-1}), respectively.

3.3.3. DCF, CBZ and TCC Extraction and Analysis

Triplicates of soil samples (5.0 g, wet weight), spiked overnight with DCF, CBZ or TCC (50 ng g^{-1}), were used to measure the recovery of each chemical in each soil. DCF, CBZ or TCC were extracted from soils employing the modified QuEChERS extraction method (Anastassiades et al., 2003). Briefly, 5 g of spiked soil was placed in a 50-ml corning plastic centrifuge tube along with 20 ng g^{-1} of internal standard. Then 10 ml of DI water plus 15 ml of acetonitrile were added to the centrifuge tube. The tube was capped and the whole mixture was vortexed for 1 min before adding the pre-mixed QuEChERS buffer (1.5 g of sodium acetate and 6 g of magnesium sulfate). The addition of the buffer facilitates separation of phases. Upon removing the cap, the tube was placed on a vortex set at low speed and the QuEChERS buffer was added slowly and continuously (Bragança, 2012) to avoid the formation of agglomerates which may compromise the extraction efficiency. Following the addition of the buffer, the tube was capped; then the content was vigorously shaken manually for 30s and vortexed at high speed for 30s. The tube was then put on a shaker (750 rpm, 3 min) before centrifugation at 5000 rpm (2 min). The supernatant (10 ml) was transferred into a vial that was then placed on an evaporating unit to dry

the extract. The dry extract was redissolved in 60 ml methanol/DI water (3:97). Triplicates of soil samples were employed for the extraction. The same extraction procedure was followed for the anaerobic microcosms except that no additional water was added for the extraction.

3.3.4. Solid Phase Extraction (SPE)

The SPE method for CBZ was the same as previously described (Thelusmond et al., 2016). DCF or TCC soil extracts were purified using Strata-X (Phenomenex, Torrance, CA, USA) cartridges, preconditioned with 5 ml of methanol followed by 5 ml of DI water. Then, the redissolved extracts (60 ml) were loaded on the cartridges; the containers were rinsed with 10 ml of DI water and the rinse was also loaded on the cartridges. The cartridges were washed with an additional 5 ml DI water before drying under vacuum for 30 min. DCF or TCC was subsequently eluted with 5 ml methanol twice. Finally, the eluates were completely dried under a gentle stream of nitrogen and reconstituted with 1 ml methanol. The final volume was used to performed liquid chromatography tandem mass spectrometry (LC/MS/MS) (as described below).

3.3.5. LC-ESI-MS/MS

Liquid chromatography was performed on a Shimadzu HPLC system (CBZ) equipped with an autosampler and a binary pump. CBZ was separated using Supelco Ascentis $^{(R)}$ Express C18 column (50mm x2.1mm, 2.7µm) at 50^{o}C. The mobile phases consisted of 0.1% formic acid in milliQ water (A) and acetonitrile (B). A solvent gradient was performed with the aforementioned mobile phases as follows: at 0 min 90% solvent A and 10% solvent B; from 0-0.5 min, solvents A and B remain constant; from 0.5-2 min, solvent B increases to 99% held and solvent A decreases to 1%; from 2-3 min, solvent A and B were held constant 1% and 99%, respectively; from 3-3.01, solvents A increases to 90% and solvent B decreases to 10%; from 3.01-4 min, both solvents A and B were held at 90% and 10%, respectively. DCF and TCC were analyzed

using Waters ACQUITY Ultra Performance LC (UPLC) system comprising also an autosampler and a binary pump. The mobile phases A and B were the same as those used for CBZ. Two separate linear mobile phase gradients were established for DCF and TCC. For DCF, the following gradient was applied: at 0 min, A/B=99:1 (v/v); 1 min A/B=99:1 (v/v); at 2 min, A/B=1:99 (v/v); at 4 min, A/B= 1:99 (v/v); at 4.01 min, A/B=99:1 (v/v); at 5 min, A/B= 99:1 (v/v). The solvent gradient used for TCC was as follows: at 0 min, A/B=99:1 (v/v); at 0.5 min, A/B=99:1 (v/v); at 2 min, A/B= 30:70 (v/v); at 3 min, A/B= 1:99 (v/v); at 4 min, A/B= 1:99 (v/v); at 4.01 min, A/B= 99:1 (v/v); at 5 min, A/B=99:1 (v/v). The equilibration time was set to 0.1 min for the column. The injection volume was 10 µl and the flow rate 0.3 ml/min for all the compounds. The LC system was used in tandem with Waters Quattro Micro or Waters TQ-D operated with electrospray ionization and Mass Lynx version 4.1. CBZ was ionized in positive mode whereas DCF and TCC were ionized in negative mode. The optimized tune page settings parameters along with precursor and product ions are shown (Supplementary Table 3.1).

3.3.6. DNA Extraction, 16S rRNA Gene Amplicon Sequencing and Mothur Analysis

DNA was extracted (0.3 g soil subsample from each microcosm) using the Power Soil DNA extraction kit (Mo Bio Laboratories, Inc. Carlsbad, CA) following the manufacturer's instructions. DNA was only extracted from the aerobic soil studies (as limited removal was noted in the anaerobic microcosms). For each chemical, DNA was extracted from triplicate sample microcosms and live control microcosms (no PPCP) at two time points (day 3 and 7 for DCF, day 21 and 40 for CBZ and day 21 and 50 for TCC). Total genomic DNA extracts were submitted for high throughput 16S rRNA gene amplicon sequencing following the protocol described elsewhere (Caporaso et al., 2012; Caporaso et al., 2011) at the Research Technology Support Facility (RTSF) at MSU. Illumina specific fusion primers were used to amplify the V4

region of the 16S rRNA gene and to add unique barcodes to samples in each well to enable pooling and sequencing. The amplicons were checked on 1% agarose gel, equimolar amounts of the sample were pooled to normalize results, purified and sequenced on the Illumina MiSeq Sequencing System. The amplicon sequencing data in the fastq file format were analyzed using Mothur (Schloss et al., 2009). The analysis involved the removal of barcode information, the creation of contiguous sequences (using the forward and reverse reads), the removal of errors and sequence classification. Samples were checked for the proper read length (<275 bp), ambiguous bases and homopolymer length greater than 8. These sequences were then aligned with the SILVA bacteria database (Pruesse et al., 2007) for the V4 region. Chimeras, mitochondrial and chloroplast lineage sequences were removed and then the sequences were classified into OTU's. Illumina sequencing data sets were deposited in the NCBI Sequence Read Archive under BioProject: PRJNA429625 and BioSample: SAMN08348582. Principal component analysis of the four soils in the controls and samples was performed with STAMP (Statistical Analyses of Metagenomic Profiles, software version 2.1.3.). Heatmaps for the 25 most abundant OTUs for each soil, for each chemical, were also generated with STAMP.

3.3.7. The Prediction of Inferred Metagenomes

PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (Langille et al., 2013) was used to predict the functional composition of the sample and control metagenomes from the 16S rRNA gene data. For this, all sequences were first reclassified to the Greengenes gene database (DeSantis et al., 2006) (as required by PICRUSt) and OTU biom tables were created using Mothur. Following this, normalized OTU tables were generated using PICRUSt, and these were used to create a functional metagenome prediction file for each DNA extract sequenced. The functional metagenome predictions files were analyzed

using the “categorize by function” command (level 3) using the KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathways. Only the KEGG pathways associated with metabolism were considered in the statistical analysis, which included using STAMP to detect differences in the relative proportions of the taxonomic and metagenomic profiles (Parks et al., 2014). This analysis included Welch’s two sided t-test for two groups (samples and live controls) with one filter ($p<0.05$) to generate extended error bar figures to compare taxonomic and metagenomic data for each chemical. For each chemical, the data for the four soils were considered separately. In addition, for each chemical, all four soils were considered together. Further, for each chemical, the data from both time points were considered separately and were also combined and considered together.

3.4. Results

3.4.1. Extraction Efficiency and Degradation Data

The extraction methods for DCF, CBZ and TCC yielded acceptable recoveries (97-126%) (Supplementary Table 3.2). DCF concentrations over time for the different TEAPs conditions are shown (Figure 3.1). Under aerobic conditions, DCF was rapidly degraded in all four soils within one week in the live microcosms but not in the abiotic controls (Figure 3.1A). Interestingly, at day 3, DCF was depleted in the soils (C and D) with lower organic matter (1.4, 1.9%), but was still present in the soils (A and B) with higher organic carbon matter (2.8, 2.4%), perhaps suggesting sorption may have retarded biodegradation in soils A and B. Only two soils (A and C) were examined for the other TEAP conditions and, in the majority of cases, the concentration of DCF in the live microcosms was not statistically significantly different from those in the abiotic controls (Figure 3.1 B-D). DCF concentrations were lower in the samples compared to the controls

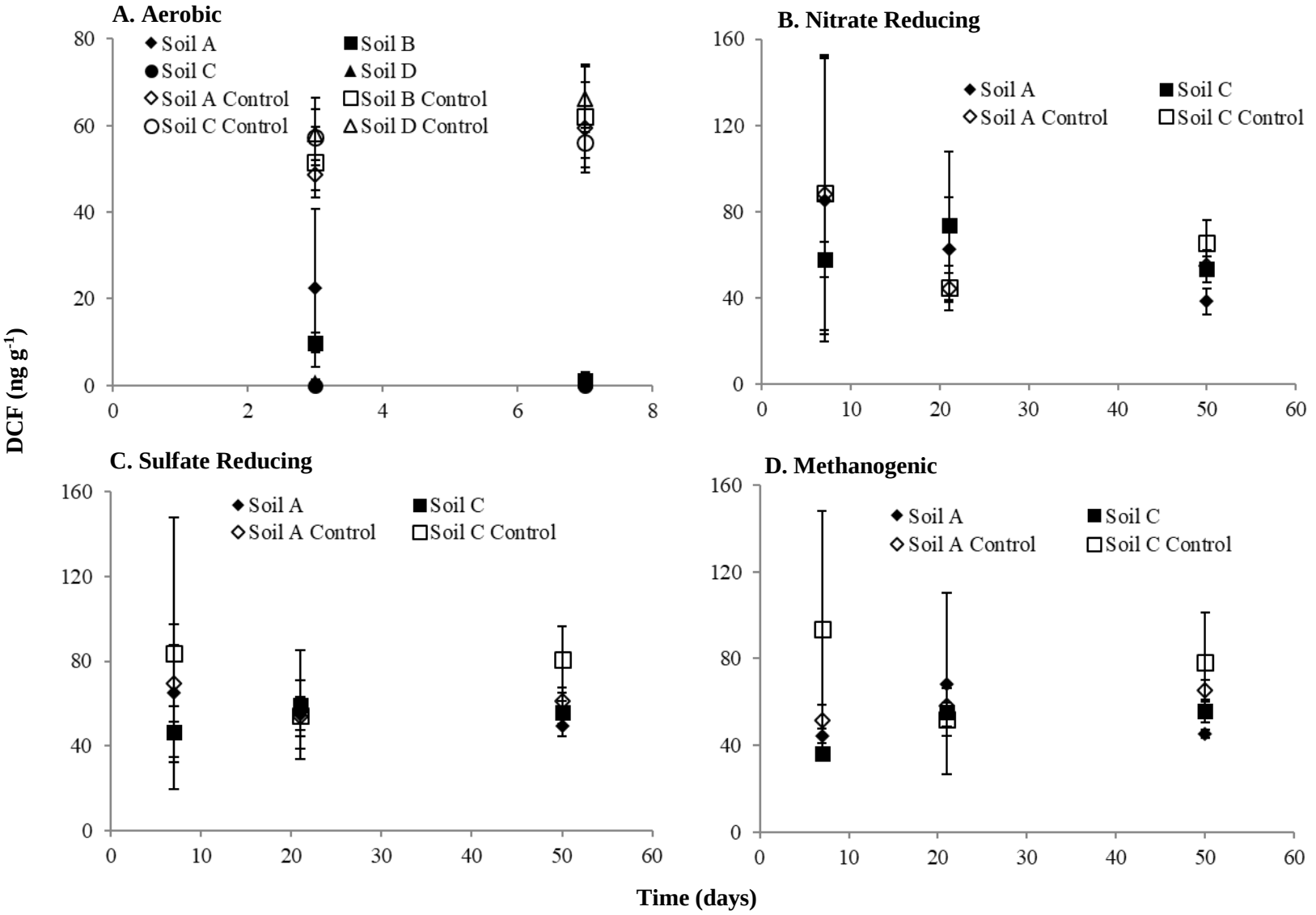


Figure 3.1 Average DCF remaining (ng g^{-1}) under different redox conditions (A-D) in four soils (A) or two soils (B, C, D) in live sample microcosms and abiotic controls. The bars represent standard deviations from two or three microcosms. All aerobic samples were significantly different from the abiotic controls at both time points. Anaerobic samples were not significantly different from the abiotic controls for treatments in B, C and D (except soil A, day 50, B, D).

(t-test, $p<0.05$) only under nitrate reducing and methanogenic conditions at one time point for one soil (day 50, soil A).

The biological degradation of CBZ and TCC was investigated only under aerobic conditions. While DCF exhibited rapid dissipation in all the aerobic soils (Figure 3.1A), a limited decrease of CBZ was noted in all four soils under aerobic conditions (Figure 3.2A). Although statistically significant differences ($p<0.05$) were found between the CBZ amended samples and abiotic controls for the majority of sampling times (except day 4, soil B), <20% removal occurred within the 40 days of incubation. Again, the soils (soils C and D) with lower organic matter exhibited lower CBZ concentrations at days 21 and 40. Estimated CBZ half-life values were greater than ~130 days (241±110, 178±6, 135±27, and 128±7 days in soils A, B, C, and D, respectively). TCC removal was similarly slow under the conditions tested (Figure 3.2B). At day 21, there was only a statistically significant difference between the samples and controls for soils A and D. Whereas, at day 50, all four soils illustrated statistically significant differences in the samples compared to the controls. Over the 50-day incubation experiment, TCC half-life values were greater than 165 days (165±68, 193±0.0, 169±53, and 190±54 days for soils A, B, C, and D, respectively). In the repeated experiment (over 70 days, data not shown), TCC half-life values in soils A, B, C, and D were similar (97±9, 146±5, 204±77, and 243±77 days).

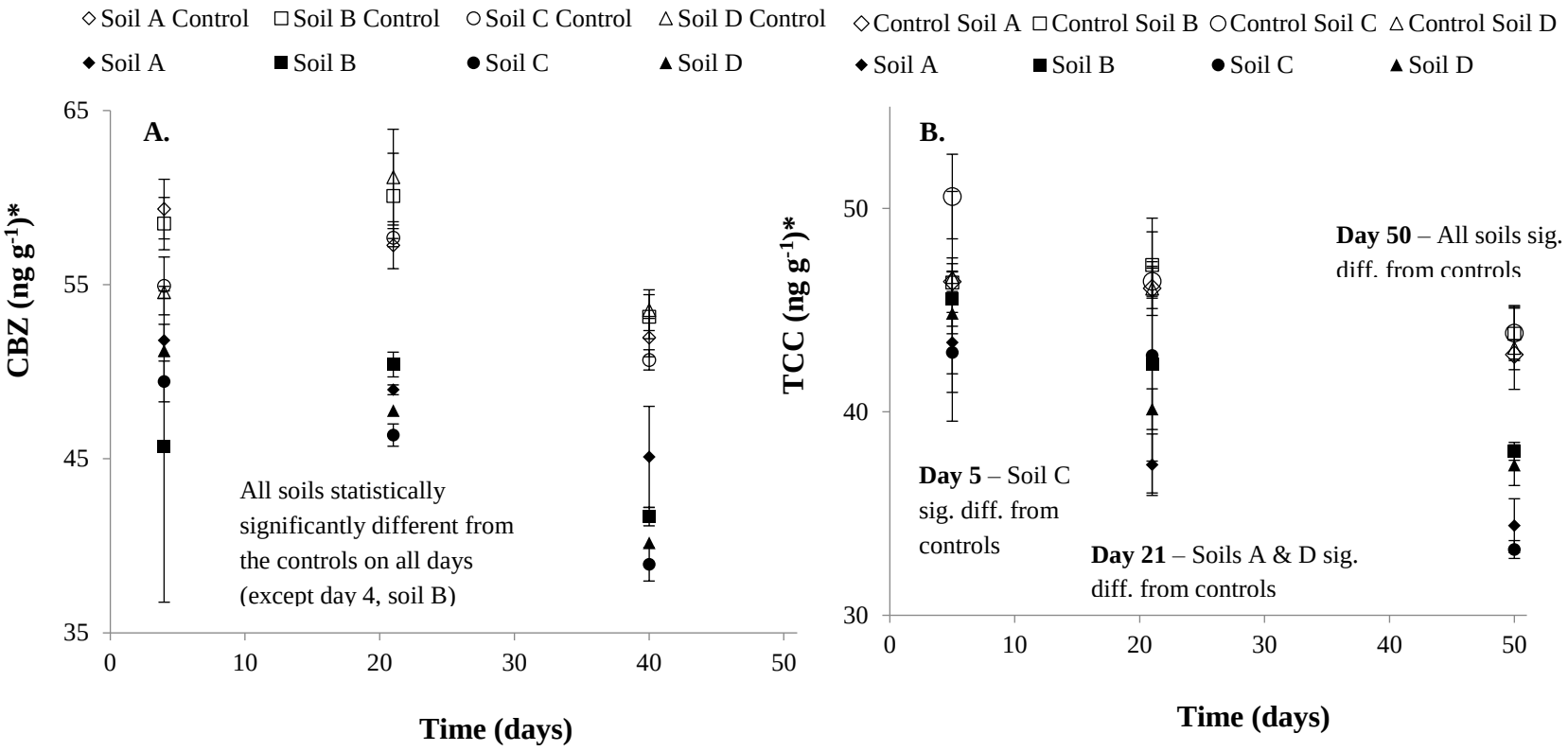


Figure 3.2 Average CBZ and TCC remaining (ng g^{-1}) under aerobic conditions in four soils in the live sample microcosms compared to abiotic controls. The bars represent standard deviations from three microcosms. The initial CBZ or TCC concentration was 50 ng/g soil, *non-zero axis.

3.4.2. Microbial Community Analysis

The principal component analysis indicated that under the majority of conditions the four soils contained different communities (as the replicates clustered together yet separately from other soils) (Figure 3.3). Interestingly, the separation between soils was more distinct for the microbial communities with or without DCF (Figure 3.3A and B) as well as for those with or without TCC (Figure 3.3E and F). In contrast, soils A, B and D clustered together for the no CBZ controls and the samples amended with CBZ (Figure 3.3 C and D), whereas soil C was clearly separated from the main cluster.

The analysis of the most abundant OTUs for each soil illustrated different trends between the soils and chemicals (Figures 3.4, 3.5 and 3.6). For soil A, the most abundant phylotypes were *Acidobacteria Gp6* and *Sphingomonadaceae* in the controls and samples for the DCF and TCC associated experiments (Figures 3.4 and 3.6), whereas only *Acidobacteria Gp 6* was dominant in the CBZ associated experiments (Figure 3.5). For soil B, *Acidobacteria Gp6* and *Sphingomonadaceae* were dominant for all three experiments (Figures 3.4, 3.5 and 3.6). For soil C, more than four phylotypes dominated the microbial communities in the DCF (Figure 3.4) and TCC (Figure 3.6) samples and controls, whereas only *Sphingomonadaceae* was dominant in the controls and samples of the CBZ experiments (Figure 3.5). Under all three conditions, for soil D, the phylotype *Acidobacteria Gp 6* was the dominant microbial community member.

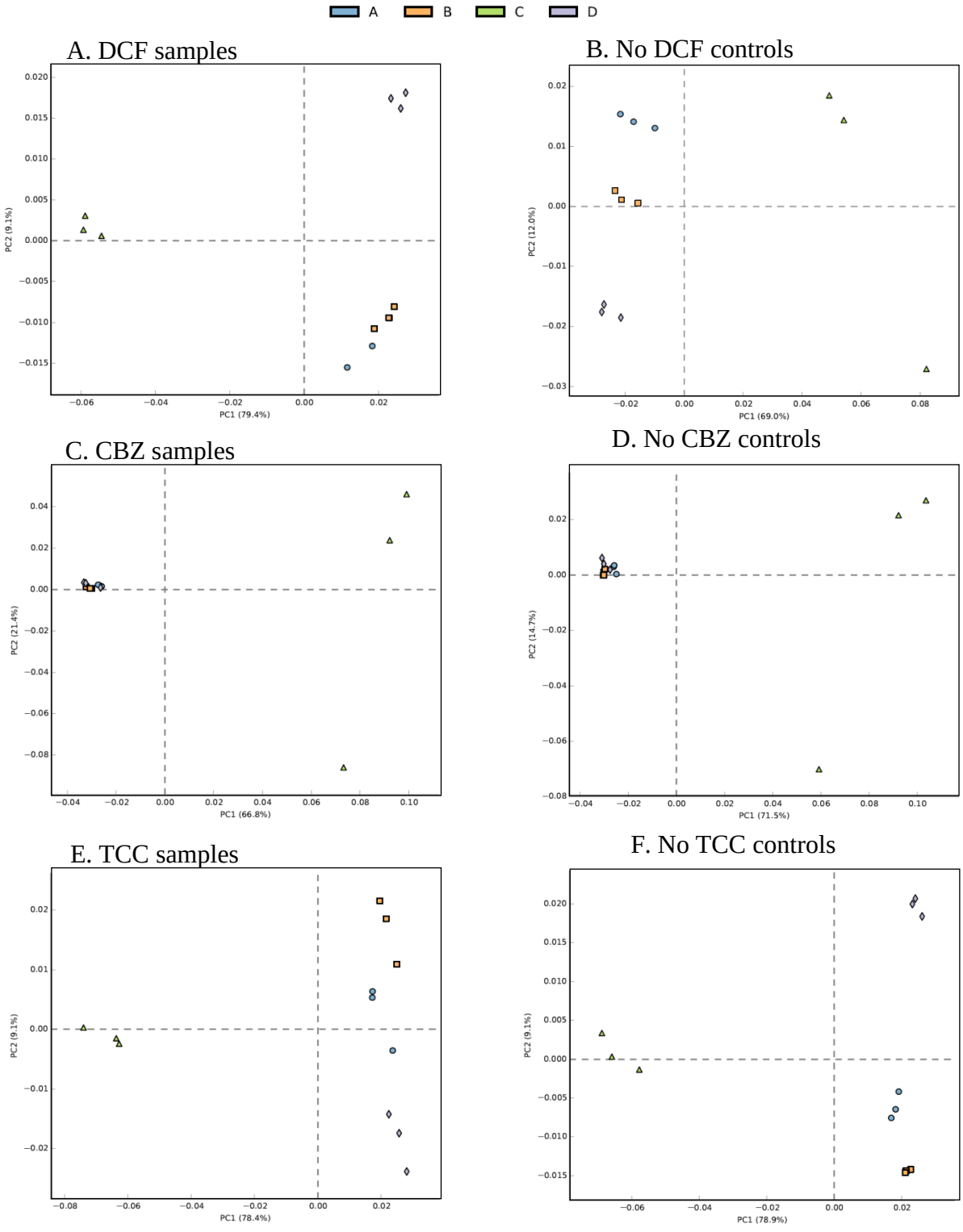


Figure 3.3 Principal component analysis of DCF amended samples (A), no DCF controls (B), CBZ amended samples (C) no CBZ controls (D), TCC amended samples (E) and no TCC controls (F) for the four soils (A, B, C and D) . Only the last time point (day7 for DCF, day 40 for CBZ and day 50 for TCC) for each was considered in the analysis.

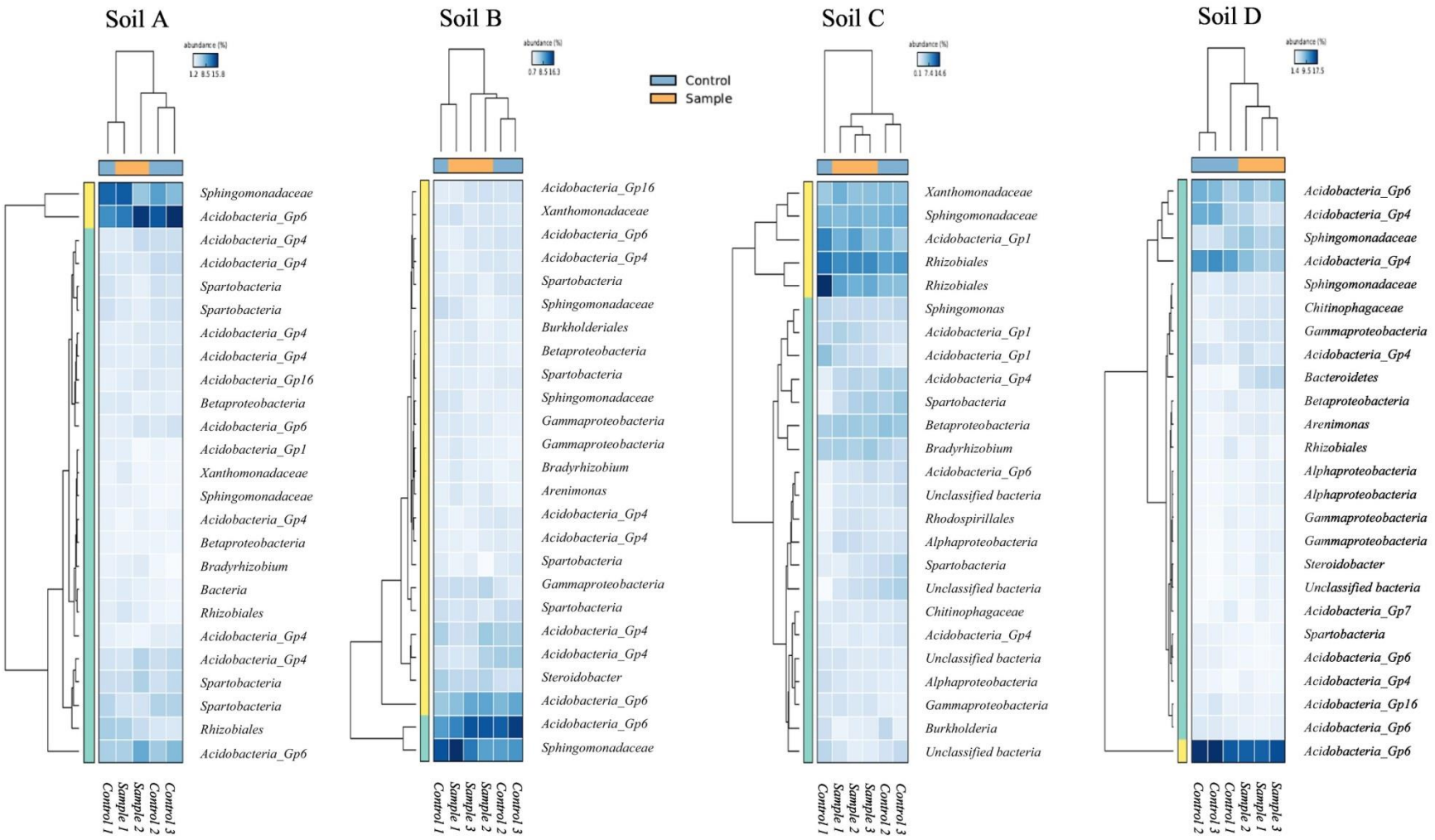


Figure 3.4 Heatmaps of the 25 most abundant OTUs (shown as the corresponding phylotype) at day 7 in soils A, B, C and D from the DCF amended samples and controls (no DCF).

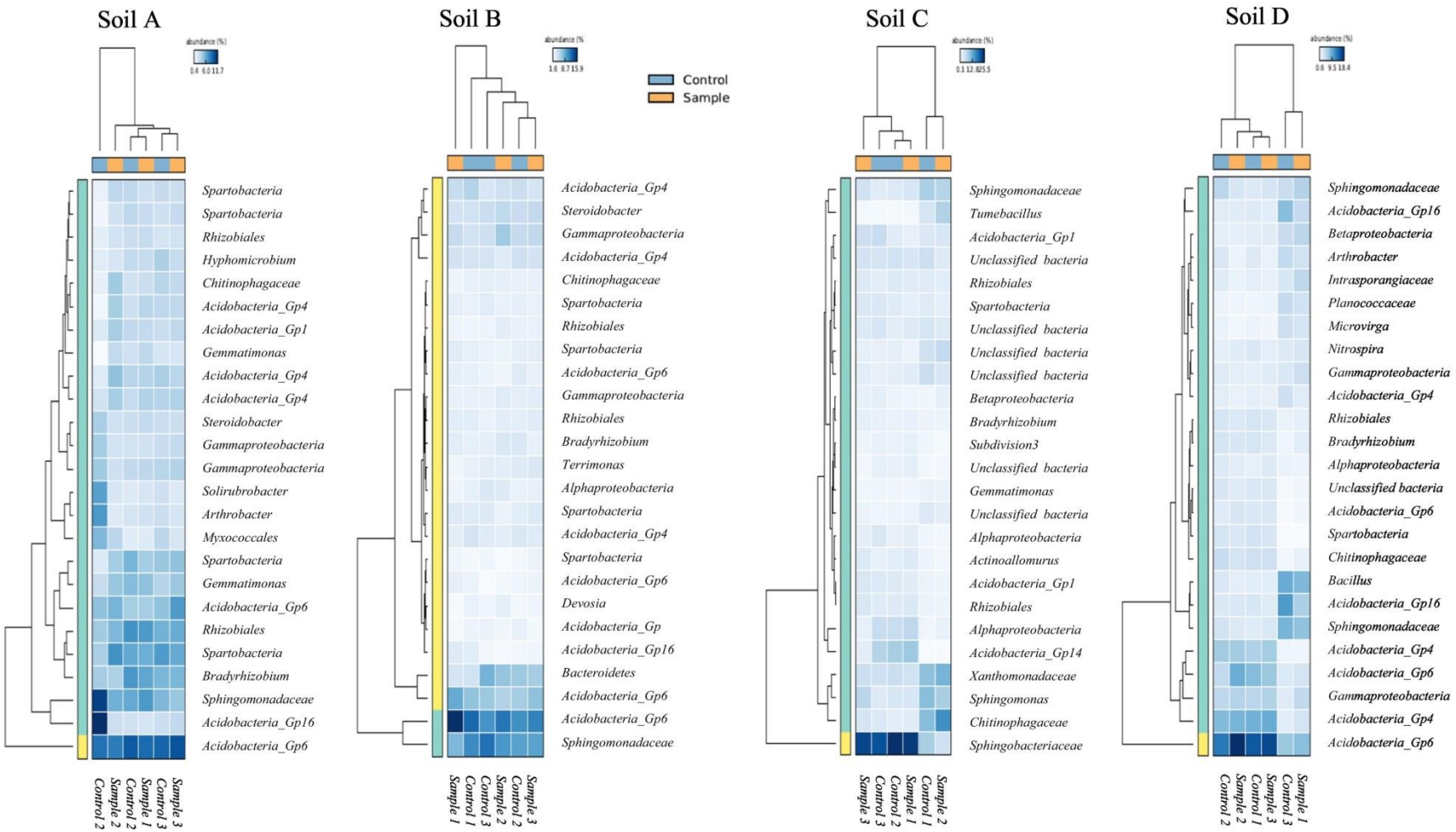


Figure 3.5 Heatmaps of the 25 most abundant OTUs (shown as the corresponding phylotype) at day 40 in soils A, B, C and D from the CBZ amended samples and controls (no CBZ).

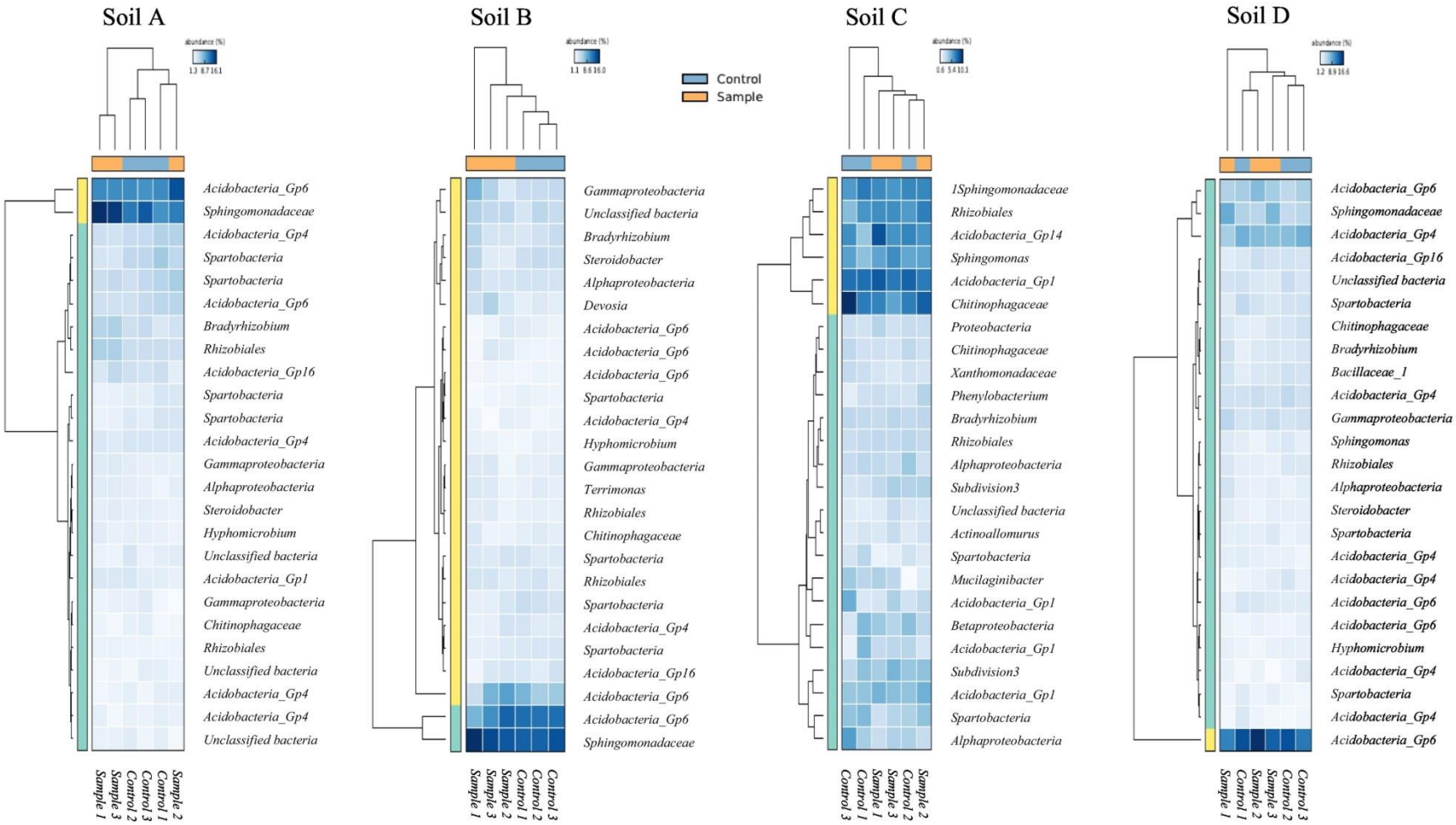


Figure 3.6 Heatmaps of the 25 most abundant OTUs (shown as the corresponding phylotype) at day 50 in soils A, B, C and D from the TCC amended samples and controls (no TCC).

3.4.3. The Effect of DCF, CBZ and TCC on OTUs

The OTUs enriched or negatively impacted upon exposure to DCF, CBZ and TCC are shown (Tables 3.1 to 3.6). In the interest of space, only the top 25 OTUs are listed for each chemical. When each soil was examined individually, no clear trends were apparent (data not shown), therefore, for each chemical, the data for all four soils were combined for the STAMP statistical analysis (comparing the live samples with the live controls). Sequencing was not performed on the anaerobic DCF samples, as limited removal was noted under these conditions. Phylotypes in the *Proteobacteria, Gemmatimonadales* and *Actinobacteria* were significantly more abundant in the microcosms amended with DCF on day 3 and 7, compared to the controls (Table 3.1). Conversely, microorganisms classifying within the *Verrucomicrobia*, *Acidobacteria*, and *Planctomycetes* were negatively impacted by DCF (Table 3.2). For CBZ, microorganisms primarily in the phyla *Bacteroidetes, Actinobacteria, Proteobacteria* and *Verrucomicrobia* were enriched on day 21 and 40 (Table 3.3). Whereas, those within the phyla including *Proteobacteria, Planctomycetes*, *Actinobacteria*, and *Firmicutes* were negatively impacted by CBZ (Table 3.4). Phylotypes that increased in abundance following exposure to TCC on days 21 and 50 primarily classified within the *Actinobacteria* followed by *Proteobacteria* (Table 3.5). Those that experienced a decrease following exposure to TCC included microorganisms within the *Proteobacteria*, and *Acidobacteria*, as well as unclassified bacteria (Table 3.6). The phylotypes that increased in abundance during DCF, CBZ or TCC biodegradation are likely benefiting in some way (carbon, nitrogen or energy source) from the removal of these chemicals.

Table 3. 1 Phylotypes with a statistically significantly greater level of abundance in all DCF amended soils compared to the controls by both day 3 and day 7 (only the top 25 are shown).

Phylum	**Class**	**Order**	**Family**	**Genus**
Unclassified	-	-	-	-
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces*
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonadales*	*Gemmatimonadaceae*	*Gemmatimonas*
Bacteroidetes	*Sphingobacteria*	*Sphingobacteriales*	-	-
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces*
Actinobacteria	*Actinobacteria*	*Acidimicrobiales*	-	-
Proteobacteria	*Deltaproteobacteria*	*Bdellovibrionales*	*Bacteriovoracaceae*	*Peredibacter*
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonadales*	*Gemmatimonadaceae*	*Gemmatimonas*
Unclassified	-	-	-	-
Unclassified	-	-	-	-
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonadales*	*Gemmatimonadaceae*	*Gemmatimonas*
Unclassified	-	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	-	-
Actinobacteria	*Actinobacteria*	-	-	-
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	-	-
Unclassified	-	-	-	-
Actinobacteria	*Actinobacteria*	*Solirubrobacterales*	*Solirubrobacteraceae*	*Solirubrobacter*
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	*Polyangiaceae*	*Byssovorax*
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	-	-
Acidobacteria	*Acidobacteria Gp5*	*Acidobacteria Gp5 order incertae sedis*	*Acidobacteria Gp5 family incertae sedis*	*Gp5*
Proteobacteria	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	-
Actinobacteria	*Actinobacteria*	*Acidimicrobiales*	*Acidimicrobiaceae*	*Ilumatobacter*
Firmicutes	*Bacilli*	*Bacillales*	*Bacillaceae 1*	*Bacillus*
Bacteroidetes	*Sphingobacteria*	*Sphingobacteriales*	-	-
Proteobacteria	-	-	-	-

- denotes unclassified (Tables 3.1-3.6)

Table 3. 2 Phylotypes with a statisitically significantly lower level of abundance in all DCF amended soils compared to the controls by both day 3 and day 7 (only the top 25 are shown).

Phylum	**Class**	**Order**	**Family**	**Genus**
Verrucomicrobia	*Spartobacteria*	*Spartobacteria order incertae sedis*	*Spartobacteria family incertae sedis*	*Spartobacteria genera incertae sedis*
Acidobacteria	*Acidobacteria Gp4*	*Acidobacteria Gp4 order incertae sedis*	*Acidobacteria Gp4 family incertae sedis*	*Gp4*
Verrucomicrobia	*Spartobacteria*	*Spartobacteria order incertae sedis*	*Spartobacteria family incertae sedis*	*Spartobacteria genera incertae sedis*
Verrucomicrobia	*Spartobacteria*	*Spartobacteria order incertae sedis*	*Spartobacteria family incertae sedis*	*Spartobacteria genera incertae sedis*
Verrucomicrobia	*Spartobacteria*	*Spartobacteria order incertae sedis*	*Spartobacteria family incertae sedis*	*Spartobacteria genera incertae sedis*
Acidobacteria	*Acidobacteria Gp6*	*Acidobacteria Gp6 order incertae sedis*	*Acidobacteria Gp6 family incertae sedis*	*Gp6*
Verrucomicrobia	*Opitutae*	*Opitutales*	*Opitutaceae*	*Opitutus*
Verrucomicrobia	*Spartobacteria*	*Spartobacteria order incertae sedis*	*Spartobacteria family incertae sedis*	*Spartobacteria genera incertae sedis*
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	-
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	*Gemmata*
Acidobacteria	*Acidobacteria Gp16*	*Acidobacteria Gp16 order incertae sedis*	*Acidobacteria Gp16 family incertae sedis*	*Gp16*
Acidobacteria	*Acidobacteria Gp3*	*Acidobacteria Gp3 order incertae sedis*	*Acidobacteria Gp3 family incertae sedis*	*Gp3*
unclassified	-	-	-	-
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	*Blastopirellula*
unclassified	-	-	-	-
Verrucomicrobia	-	-	-	-
unclassified	-	-	-	-
Proteobacteria	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	-
unclassified	-	-	-	-
Acidobacteria	*Acidobacteria Gp6*	*Acidobacteria Gp6 order incertae sedis*	*Acidobacteria Gp6 family incertae sedis*	*Gp6*
unclassified	-	-	-	-

Table 3. 3 Phylotypes with a statistically significantly greater level of abundance in all CBZ amended soils compared to the controls by both day 21 and day 40 (only the top 25 are shown).

Phylum	**Class**	**Order**	**Family**	**Genus**
Unclassified	-	-	-	-
Bacteroidetes	*Bacteroidetes incertae sedis*	*Bacteroidetes incertae sedis order incertae sedis*	*Bacteroidetes incertae sedis family incertae sedis*	*Ohtaekwangia*
Proteobacteria	*Gammaproteobacteria*	*Legionellales*	*Legionellaceae*	*Legionella*
Actinobacteria	*Actinobacteria*	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	-	-
OP11	*OP11 class incertae sedis*	*OP11 order incertae sedis*	*OP11 family incertae sedis*	*OP11 genus incertae sedis*
Acidobacteria	*Acidobacteria Gp6*	*Acidobacteria Gp6 order incertae sedis*	*Acidobacteria Gp6 family incertae sedis*	*Gp6*
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	-	-
Bacteroidetes	*Sphingobacteria*	*Sphingobacteriales*	*Chitinophagaceae*	*Terrimonas*
WS3	*WS3 class incertae sedis*	*WS3 order incertae sedis*	*WS3 family incertae sedis*	*WS3 genus incertae sedis*
Unclassified	-	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	*Hyphomicrobium*
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	*Singulisphaera*
Bacteroidetes	*Sphingobacteria*	*Sphingobacteriales*	*Chitinophagaceae*	-
Unclassified	-	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	-	-
Unclassified	-	-	-	-
Proteobacteria	-	-	-	-
Verrucomicrobia	*Subdivision3*	*Subdivision3 order incertae sedis*	*Subdivision3 family incertae sedis*	*3 genus incertae sedis*
Verrucomicrobia	-	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Brucellaceae*	*Ochrobactrum*
Unclassified	-	-	-	-
Unclassified	-	-	-	-
Verrucomicrobia	*Spartobacteria*	*Spartobacteria order incertae sedis*	*Spartobacteria family incertae sedis*	*Spartobacteria genera incertae sedis*
Verrucomicrobia	-	-	-	-

Table 3. 4 Phylotypes with a statisitically significantly lower level of abundance in all CBZ amended soils compared to the controls by both day 21 and day 40 (only the top 25 are shown).

Phylum	**Class**	**Order**	**Family**	**Genus**
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	*Planctomyces*
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	-	-
Proteobacteria	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	-
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Micromonosporaceae*	-
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	-
Proteobacteria	-	-	-	-
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	-
Actinobacteria	*Actinobacteria*	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	*Rhodospirillaceae*	*Rhodocista*
Proteobacteria	*Deltaproteobacteria*	-	-	-
Actinobacteria	*Actinobacteria*	*Solirubrobacterales*	-	-
unclassified	-	-	-	-
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	-	-
unclassified	-	-	-	-
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonadales*	*Gemmatimonadaceae*	*Gemmatimonas*
Armatimonadetes	*Armatimonadetes gp4 class incertae sedis*	*Armatimonadetes gp4 order incetae sedis*	*Armatimonadetes gp4 family incetae sedis*	*Armatimonadetes gp4*
unclassified	-	-	-	-
Proteobacteria	*Deltaproteobacteria*	-	-	-
unclassified	-	-	-	-
Firmicutes	*Bacilli*	*Bacillales*	*Thermoactinomycetaceae 1*	*Thermoflavimicrobium*
Firmicutes	*Bacilli*	*Bacillales*	*Paenibacillaceae 1*	*Paenibacillus*
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	*Isosphaera*
Planctomycetes	*Planctomycetacia*	*Planctomycetales*	*Planctomycetaceae*	-
Proteobacteria	*Gammaproteobacteria*	*Legionellales*	*Coxiellaceae*	*Aquicella*
Acidobacteria	*Acidobacteria Gp4*	*Acidobacteria Gp4 order incertae sedis*	*Acidobacteria Gp4 family incertae sedis*	*Gp4*

Table 3. 5 Phylotypes with a statistically significantly greater level of abundance in all TCC amended soils compared to the controls by both day 21 and day 50 (only the top 25 are shown).

Phylum	**Class**	**Order**	**Family**	**Genus**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Intrasporangiaceae*	-
Firmicutes	*Bacilli*	*Bacillales*	*Planococcaceae*	-
Actinobacteria	*Actinobacteria*	*Solirubrobacterales*	*Solirubrobacteraceae*	*Solirubrobacter*
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	-	-
Actinobacteria	*Actinobacteria*	-	-	-
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Massilia*
unclassified	-	-	-	-
Actinobacteria	*Actinobacteria*	*Solirubrobacterales*	-	-
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Micromonosporaceae*	-
unclassified	-	-	-	-
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Pseudonocardia*
unclassified	-	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	*Rhizobium*
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Nocardioidaceae*	*Kribbella*
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Nocardioidaceae*	-
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	-	-
unclassified	-	-	-	-
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Cellulomonadaceae*	*Cellulomonas*
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Nocardioidaceae*	-
Actinobacteria	*Actinobacteria*	-	-	-
unclassified	-	-	-	-
Proteobacteria	-	-	-	-
unclassified	-	-	-	-
Actinobacteria	*Actinobacteria*	*Solirubrobacterales*	*Solirubrobacteraceae*	*Solirubrobacter*
Actinobacteria	*Actinobacteria*	*unclassified*	*unclassified*	*unclassified*

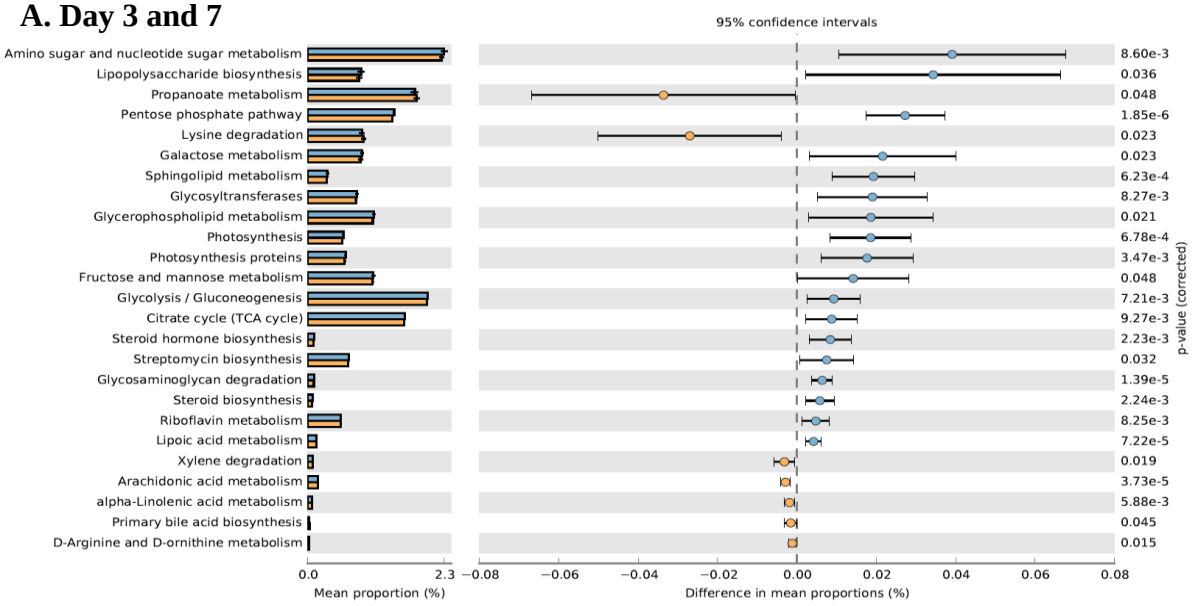
Table 3. 6 Phylotypes with a statisitically significantly lower level of abundance in all TCC amended soils compared to the controls by both day 21 and day 50 (only the top 25 are shown).

Phylum	**Class**	**Order**	**Family**	**Genus**
Verrucomicrobia	*Spartobacteria*	*Spartobacteria order incertae sedis*	*Spartobacteria family incertae sedis*	*Spartobacteria genera incertae*
Acidobacteria	*Acidobacteria Gp4*	*Acidobacteria Gp4 order incertae sedis*	*Acidobacteria Gp4 family incertae sedis*	*Gp4*
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	*Rhodospirillaceae*	-
Acidobacteria	*Acidobacteria Gp4*	*Acidobacteria Gp4 order incertae sedis*	*Acidobacteria Gp4 family incertae sedis*	*Gp4*
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
Acidobacteria	*Acidobacteria Gp4*	*Acidobacteria Gp4 order incertae sedis*	*Acidobacteria Gp4 family incertae sedis*	*Gp4*
Firmicutes	*Bacilli*	*Bacillales*	*Pasteuriaceae*	*Pasteuria*
unclassified	-	-	-	-
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	-	-
unclassified	-	-	-	-
Proteobacteria	*Alphaproteobacteria*	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	*Rhodospirillaceae*	-
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-
unclassified	-	-	-	-

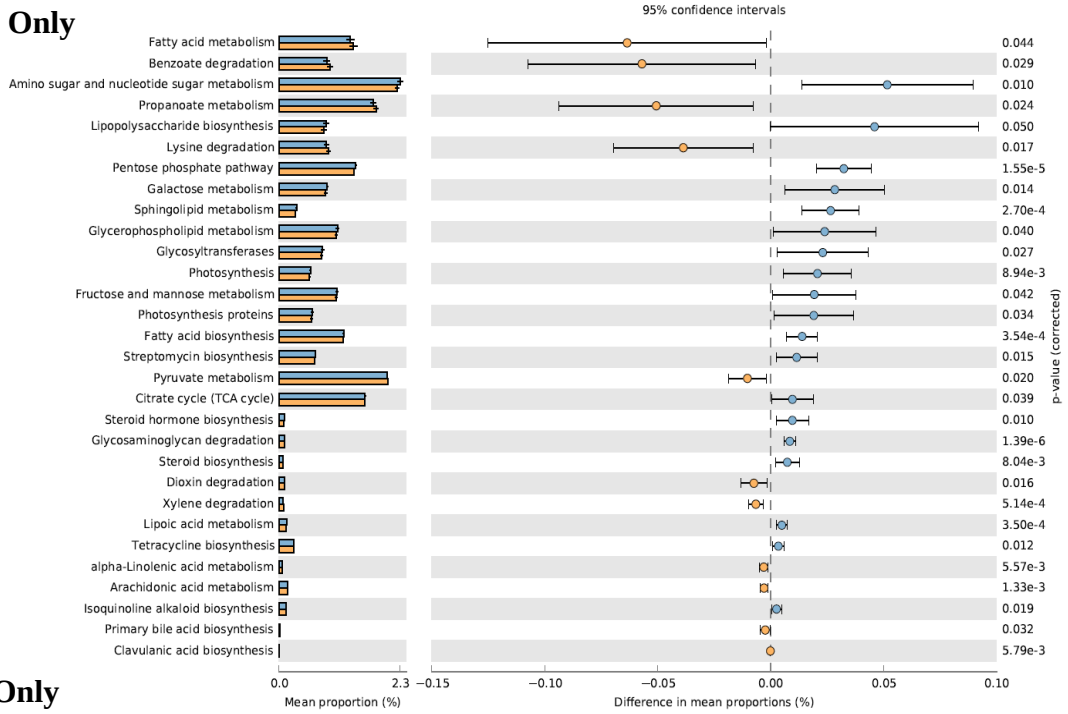
3.4.4. The Effect of DCF, CBZ and TCC on Predicted Metabolic Pathways

The PICRUSt generated data was compared between the live samples and live controls (no PPCP) to determine which pathways were impacted by the addition of these chemicals to all four soils and may therefore be putatively linked to their metabolism. As indicated previously, only the KEGG pathways associated with metabolism were investigated to enable the generation of hypotheses concerning the metabolism of these chemicals. For DCF, when data from days 3 and 7 were combined for all four soils, the genes in two pathways exhibited a high level of enrichment (Figure 3.7A), including those involved in propanoate metabolism as well as lysine degradation. When day 3 was considered separately (again, for all four soils), four pathways exhibited the most significant difference compared to the controls (Figure 3.7B). These included those two listed above as well as the pathways of fatty acid metabolism and benzoate metabolism. Additionally, the genes associated with pyruvate, dioxin and xylene degradation were enriched to a lesser extent. When day 7 was considered separately, only one pathway was significantly impacted (arachidonic acid metabolism) and the difference between the mean proportions was low compared to the results from day 3 (Figure 3.7C). The genes in many pathways were also significantly negatively impacted by the presence of DCF (Figure 3.7). When both time points were considered together, eighteen pathways were negatively impacted (Figure 3.7A) and when they were considered separately, nineteen (Day 3, Figure 3.7B) and four (Day 7, Figure 3.7C) pathways were negatively impacted.

A. Day 3 and 7



B. Day 3 Only



C. Day 7 Only

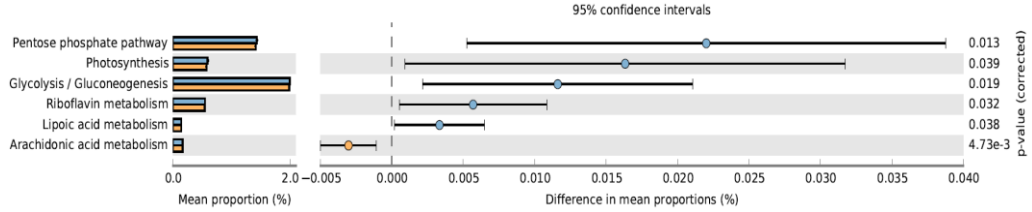
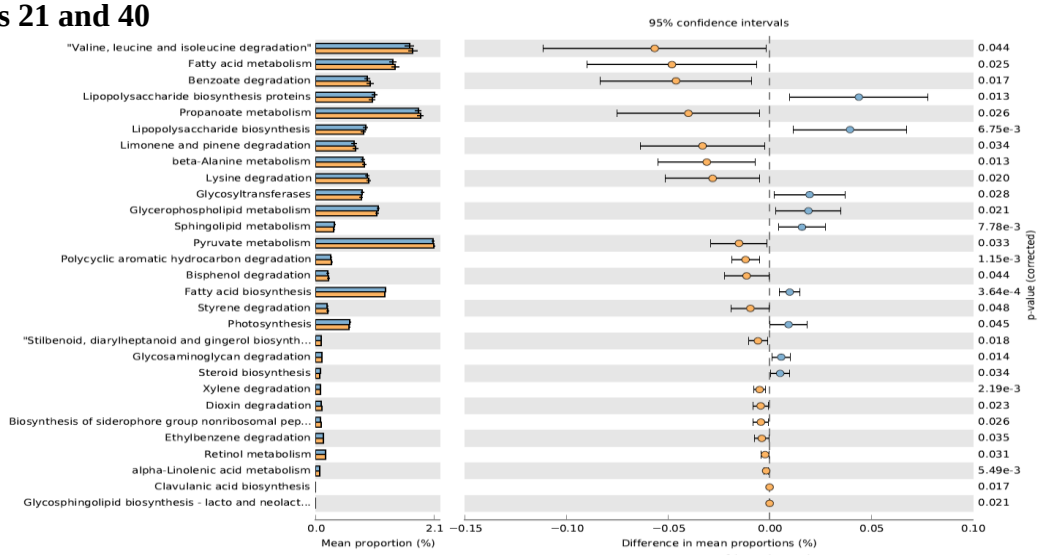


Figure 3.7 Statistically significant differences in the metabolism pathways between the samples amended with DCF and the live controls (no DCF) for all four soils at both time points (A), day 3 only (B) and day 7 only (C). The points to the left of the dashed line indicate greater values in the sample microcosms compared to the controls (no PPCP). Conversely, those to the right indicate the opposite trend.

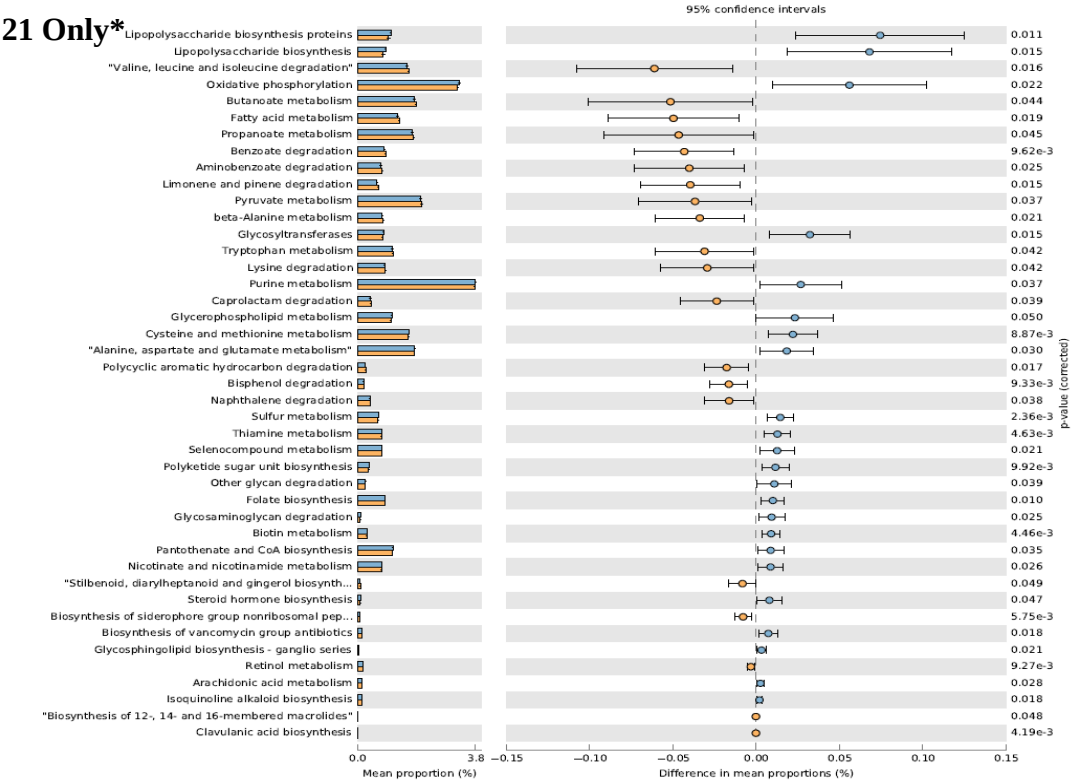
The results for CBZ were in sharp contrast to those from DCF. When both time points were analyzed together, no pathways were significantly impacted by the presence of CBZ. When only day 21 was considered, the genes in one pathway (taurine and hypotaurine metabolism) were negatively impacted (data not shown). When only day 40 was considered, the genes in one pathway (arachidonic acid metabolism) were positively impacted (data not shown).

For TCC, the data was again examined for all four soils for both days together and then separately (Figure 3.8). As TCC concentrations were not significantly different between the samples and abiotic controls for day 21 for soils B and C, those data were not included in the analysis. When both time points were considered together, eighteen pathways exhibited an increased number of genes in the samples compared to the controls (Figure 3.8A). The dominant pathways in this group included those involved in valine, leucine and isoleucine degradation, fatty acid metabolism, propanoate metabolism, limonene and pinene degradation, alanine metabolism, lysine degradation, pyruvate metabolism, polycyclic aromatic hydrocarbon (PAH) degradation and styrene degradation. In contrast, nine pathways were negatively impacted when both time points were considered together. When the data were separated with time, eighteen pathways were also positively impacted at day 21 (Figure 3.8B). In contrast at day 50, only six were positively impacted (phenylalanine metabolism, PAH degradation, dioxin degradation, xylene degradation, arachidonic acid metabolism and linolenic acid metabolism) (Figure 3.8C).

A. Days 21 and 40



B. Day 21 Only*



C. Day 50 Only

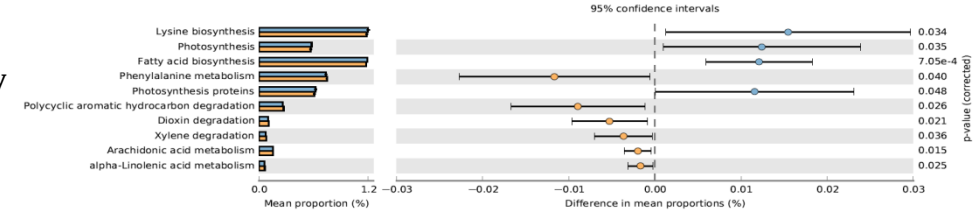


Figure 3.8 Statistically significant differences in the metabolism pathways between the samples amended with TCC and the live controls (no TCC) for all four soils at both time points (A), day 21 only (B*) and day 50 only (C). *Soils B and C were not included in the analysis. The points to the left of the dashed line indicate greater values in the sample microcosms compared to the controls (no PPCP). Conversely, those to the right indicate the opposite trend.

3.5. Discussion

DCF, CBZ, and TCC biodegradation and the impacts of these chemicals on the microbial communities were examined in four soils. DCF biodegradation was also explored under four TEAP conditions (aerobic, nitrate reducing, sulfate reducing, and methanogenic). The rapid degradation of DCF in soils under aerobic conditions in the current study is consistent with previous reports (Al-Rajab et al., 2010; Dalkmann et al., 2014; Grossberger et al., 2014). No decrease in concentration was observed in the abiotic controls, suggesting DCF removal was driven by soil microorganisms. In the current study, under anaerobic conditions, DCF biodegradation did not occur in the majority of conditions and this is also consistent with previous studies. For example, the recalcitrance of DCF under nitrate reducing conditions has been reported in membrane bioreactors (Langenhoff et al., 2013) and in water/sediment experiments (Barbieri et al., 2012; Koumaki et al., 2017). DCF persistence under sulfate reducing conditions was also previously reported in water/sediment experiments (Koumaki et al., 2017). Working with a methanogenic mixed culture, researchers found that DCF biodegradation ranged from 25-40% after 45 days of incubation (Tas et al., 2017). Limited research has been directed towards DCF biodegradation in soils under anaerobic conditions. One previous study reported negligible decrease of DCF over 84 days (the electron accepting conditions were not stated) (Lin and Gan, 2011). The common trend between current and previous research being rapid DCF degradation under aerobic versus under anaerobic conditions.

The slow biodegradation of CBZ in all four soils is consistent with previous reports (Dalkmann et al., 2014; Duran-Alvarez et al., 2015; Grossberger et al., 2014; Thelusmond et al., 2016; Walters et al., 2010), with previously reported CBZ half-life values of between 355 and 1624 days (Dalkmann et al., 2014), 495 days (Walters et al., 2010) and between 46 and >120

days (Li et al., 2013). Similar to CBZ, and consistent with previous research, TCC biodegradation in the four soils was also limited. TCC half-life values determined here are similar to those previously reported in soils (87-231 days) (Snyder et al., 2010; Wu et al., 2009; Ying et al., 2007). As shown here and previously, TCC and CBZ biological removal in soils takes a substantial amount of time, thus posing risks for ecosystems, movement into crops and water contamination.

The current research also addressed the impact of DCF, CBZ and TCC on the soil microbial communities. Here, some phylotypes appeared to benefit from DCF whereas others were negatively affected. From OTUs classified to the genus level, *Gemmatimonas* and *Steptomyces* were significantly enriched following DCF biodegradation. *Gammaproteobacteria* increased in abundance upon DCF exposure, consistent with previous results for river biofilms in experimental reactors (Lawrence et al., 2005). When the data sets were compared between the enriched phylotypes in the current study and isolates previously associated with DCF biodegradation (Aissaoui et al., 2017a; Aissaoui et al., 2017b; Bessa et al., 2017; Kim et al., 2017; Osorio-Lozada et al., 2008), no correlations were identified at the genus level. However, similarities were found at the order or class level between the phylotypes enriched here and DCF degrading isolates. Specifically, phylotypes in the *Actinomycetales* (isolate *Brevibacterium* belongs to this order) (Bessa et al., 2017), *Gammaproteobacteria* (isolate *Enterobacter* belongs to this class) (Aissaoui et al., 2017a) and *Alphaproteobacteria* (isolate *Sphingomonas* belongs to this class) (Kim et al., 2017) were enriched here. To our knowledge, no other reports have addressed the effects of DCF on soil microbial communities.

In previous research, six phylotypes (unclassified *Sphingomonadaceae*, *Xanthomonadaceae*, *Rhodobacteraceae*, *Sphingomonas*, *Aquicella*, and *Microvirga*) were putatively associated with

CBZ biodegradation (Thelusmond et al., 2016), however, these phylotypes were not identified in the current study. Higher CBZ concentrations (50 ng g^{-1} or 500 ng g^{-1} or 5000 ng g^{-1} soil) and shorter exposure times (day 4 and 14) were employed previously and these variables may explain the different results. Microorganisms in the phyla *Proteobacteria* and *Verrucomicrobia* were enriched in both studies (Thelusmond et al., 2016). Some similarities were found (again, at the order or class level) between the phylotypes enriched here and CBZ degrading isolates. Specifically, phylotypes in the *Actinobacteria* (isolates *Rhodococcus* and *Streptomyces* belong to this class), *Rhizobiales* (isolates *Starkeya* and *Rhizobium* belong to this order) and *Gammaproteobacteria* (isolate *Pseudomonas* belongs to this class) were enriched in the current study.

As for TCC, microorganisms in the class *Actinobacteria* were enriched in the current study during TCC removal. It is difficult to compare these findings to previous reports due to lack of data on microorganisms responsible for TCC degradation in soils. One project conducted in anaerobic digesters found *Actinobacteria* and *Clostridia* to thrive at high TCC concentrations ($<8.5 \times 10^5$ ng g^{-1}), while the microbial community was severely impacted following exposure (Carey et al., 2016). Some similarities were found (at the order or class level) between the phylotypes enriched here and isolates previously associated with TCC biodegradation. Specifically, phylotypes in the *Rhizobiales* (isolates in the genus *Ochrobactrum* belongs to this order), *Alphaproteobacteria* (isolate *Sphingomonas* belong to this class) and *Burkholderiales* (unclassified *Alcaligenaceae* belongs to this order) were enriched in the current study.

As stated previously, the genes in four pathways exhibited a high level of enrichment during DCF biodegradation (at the early time point for all four soils considered together), compared to the controls. These pathways included propanoate metabolism, lysine degradation, fatty acid

metabolism and benzoate metabolism. The data suggests that genes in these pathways are likely important for DCF metabolism. The degradation products were not examined in the current study, therefore it is difficult to predict which particular genes in these pathways were responsible. However, it is likely that these pathways are common in soils and this perhaps explains the rapid removal of this chemical.

Others have examined DCF biological transformation products and in one case, an enzyme has been associated with DCF degradation. For example, DCF biodegradation by *Enterobacter cloacae* (D16) produced five (unidentified) metabolites (Aissaoui et al., 2017b). The metabolite, 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one, was formed by *Enterobacter hormaechei* D15 (Aissaoui et al., 2017a) and was also reported in other studies (Bouju et al., 2016; Kosjek et al., 2009). When biomass from a wastewater treatment plant was exposed to DCF, up to 20 transformation products were detected (Jewell et al., 2016). Others discovered three DCF transformation products (5-hydroxydiclofenac, decarboxylated DCF, and nitroso DCF) using wastewater effluents (Poirier-Larabie et al., 2016). To our knowledge, only one enzyme have been linked to DCF biodegradation, a cytochrome P450 (from an *Actinoplanes* sp.), which was associated with the production of monohydroxylated DCF metabolites (Prior et al., 2010). Our results contribute to this literature through the hypothesis that the enzymes associated with DCF biodegradation in soils are components of commonly found biological pathways. Hence, it is expected that biological removal of DCF in soils should be an effective depletion pathway for this chemical.

In contrast to the results from DCF, the genes in only one pathway (arachidonic acid metabolism) were positively impacted (day 41) during CBZ biodegradation. These results are in contrast to previous results in our laboratory involving PICRUSt predictions of metabolic

pathways during CBZ biodegradation (Thelusmond et al., 2016). In that study, several xenobiotic degradation pathways (e.g. drug metabolism, chloroalkane, chloralkene) exhibited significantly different numbers of genes compared to the controls. However, that study only involved one soil and the DNA was extracted at an earlier time (day 14). It is possible that the effect of CBZ on the soil community decreases with time, explaining the lack of impact in the current work.

Research to date on CBZ metabolites and the enzymes responsible for CBZ biodegradation is limited. In batch experiments with wastewater treatment plant effluent, the metabolite epoxycarbamazepine (carbamazepine-10,11-epoxide) was generated from CBZ biodegradation (Martinez-Hernandez et al., 2016). In soil, CBZ was transformed to various intermediates including 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine-10,11-epoxide, acridone-*N*-carbaldehyde, 4-aldehyde-9-acridone, and acridine (Li et al., 2013). In experiments with endophytic bacteria (*Rhizobium radiobacter* and *Diaphorobacter nitroreducens*) and *Phragmites australis*, two CBZ biodegradation pathways were proposed (Sauvetre et al., 2018). *R. radiobacter* was proposed to be important for the transformation of CBZ to 2,3-dihydro-2,3-dihydroxycarbamazepine, 10,11-dihydroxycarbamaepine, and carbamazepine-2,3-quinone. Whereas, *D. nitroreducens* was important for CBZ to carbamazepine-10,11-epoxide, 9-acridine carboxaldehyde, acridine, 9-hydroy-acridine and acridone. Recently, a biphenyl dioxygenase from *Paraburkholderia xenovorans* LB400 was shown to rapidly oxidize CBZ generating the metabolites cis-10,11-dihydroxy-10,11-dihydrocarbamazeline and cis-2,3-dihydroxy-2,3-dihydrocarbamazepine (Aukema et al., 2017). The limited removal of CBZ in the current study and the lack of impact on the metabolic pathways indicate the enzymes responsible for the transformation of CBZ are likely not common in agricultural soils.

The enriched pathways during TCC biodegradation were dominated by valine, leucine and isoleucine degradation, fatty acid metabolism, propanoate metabolism, limonene and pinene degradation, alanine metabolism, lysine degradation, pyruvate metabolism, PAH degradation and styrene degradation. Overall, the results suggest that the genes involved in the degradation of simple substrates (e.g. amino acids) and complex substrates (PAH, dioxin) may be associated with the metabolism of TCC in the soils studied. Comparing these results to those from the literature is challenging because there is currently limited information regarding the genes or enzymes associated with TCC biodegradation. There have been several reports identifying transformation products. For example, in systems inoculated with sewage sludge, TCC biodegradation produced chloroaniline intermediates (Gledhill, 1975). The major metabolites of TCC biodegradation by *Sphingomonas* sp. strain YL-JM2C were 3,4-dichloroaniline, 4-chloroaniline and 4-chlorocatechol and the authors speculated that an amidohydrolase was responsible (Mulla et al., 2016). A reactor inoculated with activated sludge also produced 4-chloroaniline during TCC biodegradation (Armstrong et al., 2018). To date, only one enzyme has been linked to the transformation of this chemical. Specifically, an amidase gene (*tccA*) from *Ochrobactrum* sp. TCC-2 was linked to the hydrolysis of the two amide bonds of TCC (Yun et al., 2017).

In summary, DCF aerobic biodegradation was rapid (< 7 days) and was associated with KEGG pathways commonly present in soils. In contrast, DCF was persistent under the majority of other redox conditions investigated. The phylotypes *Gemmatimonas* and *Streptomyces* and others within the phyla *Proteobacteria* and *Actinobacteria* were significantly more abundant during aerobic DCF biodegradation in the samples compared to the live controls (no DCF).

CBZ and TCC aerobic biodegradation occurred, but was slow (half-life values being > ~130 and >165 days for CBZ and TCC). For CBZ, phylotypes in the *Bacteroidetes, Actinobacteria, Proteobacteria* and *Verrucomicrobia* were enriched. Similarly, those in the *Actinobacteria* and *Proteobacteria* increased in abundance following exposure to TCC. One hypothesis being these microorganisms are involved in the biodegradation of these compounds, as they are benefiting from their removal. There was a limited impact of CBZ on the KEGG metabolic pathways examined. TCC removal was associated with an increase in genes associated with the degradation of simple substrates (e.g. amino acids) as well as complex substrates (e.g. PAH, dioxin). Overall, these results suggest even low (ppb) concentrations of DCF, CBZ and TCC significantly affected the soil microbial communities. The recalcitrant nature of TCC and CBZ indicate agricultural soils receiving biosolids could accumulate these chemicals, representing risks to groundwater, surface waters and uptake into the food chain.

APPENDIX

Appendix

Supplementary Table 3.1 Precursor, product ions and optimized MS/MS parameters for DCF, CBZ, and TCC quantification.

Compound	Precursor ion> product ion	CPV(kV)[a]	CV (V)[b]	CE (V)[c]	ST (°C)[d]	DT (°C)[e]	CGF(L/hr)[f]	DGF(L/hr)[g]	CoGF (ml/min)[h]
DCF	294>214	2.5	28	17	130	350	20	800	0.15
DCF-d_4	294>250	2.5	28	17	130	350	20	800	0.15
CBZ	237>194	3.6	22	22	150	350	20	800	2
CBZ-d_{10}	247>204	3.6	22	22	150	350	20	800	2
TCC	313>160	2.5	34	16	130	350	20	800	0.15
TCC-$^{13}C_6$	319>160	2.5	34	17	130	350	20	800	0.15

a: capillary voltage; b: cone voltage; c: collision energy; d: source temperature; e: desolvation temperature; f: collision gas flow; g: desolvation gas flow; h: collision gas flow.

Supplementary Table 3.2 DCF, CBZ and TCC average percent recovery (%) (n=3) in soils A, B, C, and D.

Soil	DCF	CBZ	TCC
A	97±16	126±9	93±0.3
B	111 ±18	126±8	117±3
C	110±32	117±2	97±6
D	106±4	116±0.7	101±2

REFERENCES

REFERENCES

Ahn, K.C., Zhao, B., Chen, J., Cherednichenko, G., Sanmarti, E., Denison, M.S., et al. 2008. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens. Environmental Health Perspectives. 116, 1203-1210.

Aissaoui, S., Ouled-Haddar, H., Sifour, M., Harrouche, K., Sghaier, H. 2017a. Metabolic and co-metabolic transformation of diclofenac by *Enterobacter hormaechei* D15 isolated from activated sludge. Current Microbiology. 74, 381-388.

Aissaoui, S., Sifour, M., Ouled-Haddar, H., Benguedouar, L., Lahouel, M. 2017b. Toxicity assessment of diclofenac and its biodegradation metabolites toward mice. Toxicology and Environmental Health Sciences. 9, 284-290.

Al-Rajab, A.J., Sabourin, L., Lapen, D.R., Topp, E. 2010. The non-steroidal anti-inflammatory drug diclofenac is readily biodegradable in agricultural soils. Science of the Total Environment. 409, 78-82.

Albero, B., Sanchez-Brunete, C., Miguel, E., Aznar, R., Tadeo, J.L. 2014. Determination of selected pharmaceutical compounds in biosolids by supported liquid extraction and gas chromatography-tandem mass spectrometry. Journal of Chromatography A. 1336, 52-58.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International. 86, 412-431.

Armstrong, D.L., Lozano, N., Rice, C.P., Ramirez, M., Torrents, A. 2018. Degradation of triclosan and triclocarban and formation of transformation products in activated sludge using benchtop bioreactors. Environmental Resources. 161, 17-25.

Aukema, K.G., Escalante, D.E., Maltby, M.M., Bera, A.K., Aksan, A., Wackett, L.P. 2017. In silico identification of bioremediation potential: carbamazepine and other recalcitrant personal care products. Environmental Science & Technology. 51, 880-888.

Azzouz, A., Ballesteros, E. 2012. Combined microwave-assisted extraction and continuous solid-phase extraction prior to gas chromatography-mass spectrometry determination of pharmaceuticals, personal care products and hormones in soils, sediments and sludge. Science of the Total Environment. 419, 208-215.

Barbieri, M., Carrera, J., Ayora, C., Sanchez-Vila, X., Licha, T., Nodler, K., et al. 2012. Formation of diclofenac and sulfamethoxazole reversible transformation products in aquifer material under denitrifying conditions: Batch experiments. Science of the Total Environment. 426, 256-263.

Bessa, V.S., Moreira, I.S., Tiritan, M.E., Castro, P.M.L. 2017. Enrichment of bacterial strains for the biodegradation of diclofenac and carbamazepine from activated sludge. International Biodeterioration & Biodegradation. 120, 135-142.

Bevans, H.E., Goodbred, S.L., Miesner, J.F., Watkins, S.A., Gross, T.S., Denslow, N.D., et al. 1996. Synthetic organic compounds and carp endocrinology and histology in Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, 1992 and 1995. U.S. Geological Survey, Water-Resources Investigations Report., 96-4266.

Bouju, H., Nastold, P., Beck, B., Hollender, J., Corvini, P.F.X., Wintgens, T. 2016. Elucidation of biotransformation of diclofenac and 4 ' hydroxydiclofenac during biological wastewater treatment. Journal of Hazardous Materials. 301, 443-452.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME Journal. 6, 1621-1624.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings National Academy of Sciences of the United States of America.108, 4516-4522.

Carey, D.E., Zitomer, D.H., Hristova, K.R., Kappell, A.D., McNamara, P.J. 2016. Triclocarban influences antibiotic resistance and alters anaerobic digester microbial community structure. Environmental Science & Technology. 50, 126-134.

Cha, J.M., Cupples, A.M. 2009. Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids. Water Research. 43, 2522-2530.

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

Chen, J., Ahn, K.C., Gee, N.A., Ahmed, M.I., Duleba, A.J., Zhao, L., et al. 2008. Triclocarban enhances testosterone action: a new type of endocrine disruptor? Endocrinology. 149, 1173-1179.

Chu, S., Metcalfe, C.D. 2007. Simultaneous determination of triclocarban and triclosan in municipal biosolids by liquid chromatography tandem mass spectrometry. Journal of Chromatography A. 1164, 212-218.

Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., Kroiss, H. 2005. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. Water Research. 39, 4797-4807.

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

Dalkmann, P., Siebe, C., Amelung, W., Schloter, M., Siemens, J. 2014. Does long-term irrigation with untreated wastewater accelerate the dissipation of pharmaceuticals in soil? Environmental Science & Technology. 48, 4963-4970.

Dasu, K., Lee, L.S., Turco, R.F., Nies, L.F. 2013. Aerobic biodegradation of 8:2 fluorotelomer stearate monoester and 8:2 fluorotelomer citrate triester in forest soil. Chemosphere. 91, 399-405.

Daughton, C.G., Ternes, T.A. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environmental Health Perspectives. 107, 907-938.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., et al. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology. 72, 5069-5072.

Ding, Y.J., Zhang, W.H., Gu, C., Xagoraraki, I., Li, H. 2011. Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. Journal of Chromatography A. 1218, 10-16.

Dodgen, L.K., Li, J., Parker, D., Gan, J.J. 2013. Uptake and accumulation of four PPCP/EDCs in two leafy vegetables. Environmental Pollution. 182, 150-156.

Duran-Alvarez, J.C., Prado, B., Gonzalez, D., Sanchez, Y., Jimenez-Cisneros, B. 2015. Environmental fate of naproxen, carbamazepine and triclosan in wastewater, surface water and wastewater irrigated soil-results of laboratory scale experiments. Science of the Total Environment. 538, 350-362.

Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., Xu, L. 2012. Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. Water Research. 46, 2355-2364.

Gauthier, H., Yargeau, V., Cooper, D.G. 2010. Biodegradation of pharmaceuticals by *Rhodococcus rhodochrous* and *Aspergillus niger* by co-metabolism. Science of the Total Environment. 408, 1701-1706.

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

Gledhill, W.E. 1975. Biodegradation of 3,4,4'-trichlorocarbanilide, TCC, in sewage and activated sludge. Water Research. 9, 649-654.

Gottschall, N., Topp, E., Metcalfe, C., Edwards, M., Payne, M., Kleywegt, S., et al. 2012. Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and

wheat grain, following a high single application of municipal biosolids to a field. Chemosphere. 87, 194-203.

Grossberger, A., Hadar, Y., Borch, T., Chefetz, B. 2014. Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. Environmental Pollution. 185, 168-177.

Guerra, P., Kim, M., Shah, A., Alaee, M., Smyth, S.A. 2014. Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. Science of the Total Environment. 473, 235-243.

Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, P., Routledge, E.J., et al. 1996. A Survey of estrogenic activity in United Kingdom inland waters. Environmental Toxicology and Chemistry. 15, 1993–2002.

Holling, C.S., Bailey, J.L., Heuvel, B.V., Kinney, C.A. 2012. Uptake of human pharmaceuticals and personal care products by cabbage (*Brassica campestris*) from fortified and biosolids-amended soils. Journal of Environmental Monitoring. 14, 3029-3036.

Hussain, I., Khan, M.Z., Khan, A., Javed, I., Saleemi, M.K. 2008. Toxicological effects of diclofenac in four avian species. Avian Pathology. 37, 315-321.

Jayamani, I., Cupples, A.M. 2015. Stable isotope probing and high-throughput sequencing implicate *Xanthomonadaceae* and *Rhodocyclaceae* in ethylbenzene degradation. Environmental Engineering Science. 32, 240-249.

Jewell, K.S., Falas, P., Wick, A., Joss, A., Ternes, T.A. 2016. Transformation of diclofenac in hybrid biofilm-activated sludge processes. Water Research. 105, 559-567.

Khan, B., Lee, L.S. 2010. Soil temperature and moisture effects on the persistence of synthetic androgen 17alpha-trenbolone, 17beta-trenbolone and trendione. Chemosphere. 79, 873-9.

Kim, S., Rossmassler, K., Broeckling, C.D., Galloway, S., Prenni, J., De Long, S.K. 2017. Impact of inoculum sources on biotransformation of pharmaceuticals and personal care products. Water Research. 125, 227-236.

Kosjek, T., Heath, E., Perez, S., Petrovic, M., Barcelo, D. 2009. Metabolism studies of diclofenac and clofibric acid in activated sludge bioreactors using liquid chromatography with quadrupole - time-of-flight mass spectrometry. Journal of Hydrology. 372, 109-117.

Koumaki, E., Mamais, D., Noutsopoulos, C. 2017. Environmental fate of non-steroidal anti-inflammatory drugs in river water/sediment systems. Journal of Hazardous Materials. 323, 233-241.

Kwon, L.W., Armbrust, K.L., Xia, K. 2010. Transformation of triclosan and triclocarban in soils and biosolids-applied soils. Journal of Environmental Quality. 39, 1139-1144.

Langenhoff, A., Inderfurth, N., Veuskens, T., Schraa, G., Blokland, M., Kujawa-Roeleveld, K., et al. 2013. Microbial removal of the pharmaceutical compounds ibuprofen and diclofenac from wastewater. Biomed Research International., 1-9.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., et al. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology. 31, 814-821.

Lawrence, J.R., Swerhone, G.D., Wassenaar, L.I., Neu, T.R. 2005. Effects of selected pharmaceuticals on riverine biofilm communities. Canadian Journal of Microbiology. 51, 655-669.

Li, A., Cai, R., Cui, D., Qiu, T., Pang, C.L., Yang, J.X., et al. 2013a. Characterization and biodegradation kinetics of a new cold-adapted carbamazepine-degrading bacterium, *Pseudomonas* sp CBZ-4. Journal of Environmental Sciences (China). 25, 2281-2290.

Li, J.Y., Dodgen, L., Ye, Q.F., Gan, J. 2013b. Degradation kinetics and metabolites of carbamazepine in soil. Environmental Science &Technology 47, 3678-3684.

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

Lozano, N., Rice, C.P., Ramirez, M., Torrents, A. 2013. Fate of triclocarban, triclosan and methyltriclosan during wastewater and biosolids treatment processes. Water Research. 47, 4519-4527.

Martinez-Hernandez, V., Meffe, R., Lopez, S.H., de Bustamante, I. 2016. The role of sorption and biodegradation in the removal of acetaminophen, carbamazepine, caffeine, naproxen and sulfamethoxazole during soil contact: a kinetics study. Science of the Total Environment. 559, 232-241.

Miao, X.S., Metcalfe, C.D. 2003. Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography-electrospray tandem mass spectrometry. Analytical Chemistry. 75, 3731-3738.

Miao, X.S., Yang, J.J., Metcalfe, C.D. 2005. Carbamazepine and its metabolites in wastewater and in biosolids in a municipal wastewater treatment plant. Environmental Science & Technology. 39, 7469-7475.

Middleton, J.H., Salierno, J.D. 2013. Antibiotic resistance in triclosan tolerant fecal coliforms isolated from surface waters near wastewater treatment plant outflows (Morris County, NJ, USA). Ecotoxicology and Environmental Safety. 88, 79-88.

Miller, T.R., Heidler, J., Chillrud, S.N., Delaquil, A., Ritchie, J.C., Mihalic, J.N., et al. 2008. Fate of triclosan and evidence for reductive dechlorination of triclocarban in estuarine sediments. Environmental Science & Technology. 42, 4570-4576.

Morais, S.A., Delerue-Matos, C., Gabarrell, X., Blanquez, P. 2013. Multimedia fate modeling and comparative impact on freshwater ecosystems of pharmaceuticals from biosolids-amended soils. Chemosphere. 93, 252-262.

Mulla, S.I., Hu, A.Y., Wang, Y.W., Sun, Q., Huang, S.L., Wang, H., et al. 2016. Degradation of triclocarban by a triclosan-degrading *Sphingomonas* sp strain YL-JM2C. Chemosphere. 144, 292-296.

Ogunyoku, T.A., Young, T.M. 2014. Removal of Triclocarban and Triclosan during Municipal Biosolid Production. Water Environment Research. 86, 197-203.

Osorio-Lozada, A., Surapaneni, S., Skiles, G.L., Subramanian, R. 2008. Biosynthesis of drug metabolites using microbes in hollow fiber cartridge reactors: case study of diclofenac metabolism by *Actinoplanes* species. Drug Metabolism and Disposition. 36, 234-240.

Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G. 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 30, 3123-3124.

Poirier-Larabie, S., Segura, P.A., Gagnon, C. 2016. Degradation of the pharmaceuticals diclofenac and sulfamethoxazole and their transformation products under controlled environmental conditions. Science of the Total Environment. 557, 257-267.

Popa, C., Favier, L., Dinica, R., Semrany, S., Djelal, H., Amrane, A., et al. 2014. Potential of newly isolated wild *Streptomyces* strains as agents for the biodegradation of a recalcitrant pharmaceutical, carbamazepine. Environmental Technology. 35, 3082-3091.

Prior, J.E., Shokati, T., Christians, U., Gill, R.T. 2010. Identification and characterization of a bacterial cytochrome P450 for the metabolism of diclofenac. Applied Microbiology and Biotechnology. 85, 625-633.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., et al. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Research. 35, 7188-7196.

Radjenovic, J., Jelic, A., Petrovic, M., Barcelo, D. 2009a. Determination of pharmaceuticals in sewage sludge by pressurized liquid extraction (PLE) coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Analytical and Bioanalytical Chemistry. 393, 1685-1695.

Radjenovic, J., Petrovic, M., Barcelo, D. 2009b. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Research. 43, 831-841.

Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D.R., Topp, E. 2012. Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. Science of the Total Environment. 431, 233-236.

Sauvetre, A., May, R., Harpaintner, R., Poschenrieder, C., Schroder, P. 2018. Metabolism of carbamazepine in plant roots and endophytic rhizobacteria isolated from *Phragmites australis*. Journal of Hazardous Materials. 342, 85-95.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology. 75, 7537-7541.

Shenker, M., Harush, D., Ben-Ari, J., Chefetz, B. 2011. Uptake of carbamazepine by cucumber plants--a case study related to irrigation with reclaimed wastewater. Chemosphere. 82, 905-910.

Sipahutar, M.K., Piapukiew, J., Vangnai, A.S. 2018. Efficiency of the formulated plant-growth promoting *Pseudomonas fluorescens* MC46 inoculant on triclocarban treatment in soil and its effect on *Vigna radiata* growth and soil enzyme activities. Journal of Hazardous Materials. 344, 883-892.

Sipahutar, M.K., Vangnai, A.S. 2017. Role of plant growth-promoting *Ochrobactrum* sp MC22 on triclocarban degradation and toxicity mitigation to legume plants. Journal of Hazardous Materials. 329, 38-48.

Snyder, E.H., O'Connor, G.A., McAvoy, D.C. 2010. Fate of 14C-triclocarban in biosolids-amended soils. Science of the Total Environment. 408, 2726-2732.

Spongberg, A.L., Witter, J.D. 2008. Pharmaceutical compounds in the wastewater process stream in Northwest Ohio. Science of the Total Environment. 397, 148-157.

Tas, D.O., Sari, S., Aydın, E., Topuz, E., Pehlivanoğlu-Mantaş, E. 2017. Fate and biodegradability potential of an emerging micropollutant diclofenac in subsurface environment. International Journal of Environmental Science and Technology, DOI 10.1007/s13762-017-1485-x.

Ternes, T. 2000. Pharmaceuticals and metabolites as contaminants of the aquatic environment: an overview. Abstracts of Papers American Chemical Society. 219, U622-U623.

Thelusmond, J.R., Strathmann, T.J., Cupples, A.M. 2016. The identification of carbamazepine biodegrading phylotypes and phylotypes sensitive to carbamazepine exposure in two soil microbial communities. Science of the Total Environment. 571, 1241-1252.

Vazquez-Roig, P., Andreu, V., Onghena, M., Blasco, C., Pico, Y. 2011. Assessment of the occurrence and distribution of pharmaceuticals in a Mediterranean wetland (L'Albufera, Valencia, Spain) by LC-MS/MS. Analytical and Bioanalytical Chemistry. 400, 1287-301.

Vazquez-Roig, P., Segarra, R., Blasco, C., Andreu, V., Pico, Y. 2010. Determination of pharmaceuticals in soils and sediments by pressurized liquid extraction and liquid

chromatography tandem mass spectrometry. Journal of Chromatography A. 1217, 2471-2483.

Walker, C.W., Watson, J.E., Williams, C. 2012. Occurrence of carbamazepine in soils under different land uses receiving wastewater. Journal of Environmental Quality. 41, 1263-1267.

Walters, E., McClellan, K., Halden, R.U. 2010. Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. Water Research. 44, 6011-6020.

Wu, C., Spongberg, A.L., Witter, J.D. 2009. Adsorption and degradation of triclosan and triclocarban in soils and biosolids-amended soils. Journal of Agricultural and Food Chemistry. 57, 4900-4905.

Wu, C., Spongberg, A.L., Witter, J.D., Fang, M., Ames, A., Czajkowski, K.P. 2010a. Detection of pharmaceuticals and personal care products in agricultural soils receiving biosolids application. CLEAN - Soil, Air, Water. 38, 230-237.

Wu, C., Spongberg, A.L., Witter, J.D., Fang, M., Czajkowski, K.P. 2010b. Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water. Environmental Science & Technology. 44, 6157-6161.

Xu, J., Wu, L.S., Chang, A.C. 2009. Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere. 77, 1299-1305.

Ying, G.G., Yu, X.Y., Kookana, R.S. 2007. Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. Environmental Pollution. 150, 300-305.

Yun, H., Liang, B., Kong, D.Y., Li, Z.L., Qi, G.S., Wang, A.J. 2017a. Enhanced biotransformation of triclocarban by *Ochrobactrum* sp TCC-1 under anoxic nitrate respiration conditions. Current Microbiology. 74, 491-498.

Yun, H., Liang, B., Qiu, J., Zhang, L., Zhao, Y., Jiang, J., et al. 2017b. Functional characterization of a novel amidase involved in biotransformation of triclocarban and its dehalogenated congeners in *Ochrobactrum* sp. TCC-2. Environmental Science & Technology. 51, 291-300.

Zhang, Y.J., Geissen, S.U., Gal, C. 2008. Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. Chemosphere. 73, 1151-1161.

Chapter 4:

Carbamazepine, Triclocarban and Triclosan Biodegradation and the Phylotypes and Functional Genes Associated with Xenobiotic Degradation in Four Agricultural Soils

This chapter has been submitted to a peer reviewed journal: Thelusmond, J.R., Strathmann, T.J., Cupples, A.M. Carbamazepine, triclocarban and triclosan biodegradation and the phylotypes and functional genes associated with xenobiotic degradation in four agricultural soils

4.1. Abstract

Pharmaceuticals and personal care products (PPCPs) are released into the environment due to their poor removal during wastewater treatment. Agricultural soils subject to irrigation with wastewater effluent and biosolids application constitute reservoirs for these chemicals. This study examined the impact of the pharmaceutical carbamazepine (CBZ), and the antimicrobial agents triclocarban (TCC) and triclosan (TCS) on four soil microbial communities using shotgun sequencing (HiSeq Illumina) with the overall aim of determining possible degraders as well as the functional genes related to general xenobiotic degradation. The biodegradation of CBZ and TCC was slow, with <50 % decrease during the 80-day of incubation. In contrast, TCS biodegradation was rapid, with ~ 80% removal of the parent in 25 days. *Burkholderia* and *Streptomyces* were the most abundant genera in the samples and live controls (no chemical added) in all four soils. For each chemical, when all four soils were considered together, between three and ten phylotypes were enriched in the samples compared to the live controls or vice versa. The genera of a number of previously reported CBZ, TCC or TCS degrading isolates were present. Specifically, *Rhodococcus* (CBZ), *Streptomyces* (CBZ), *Pseudomonas* (CBZ, TCC, TCS), *Sphingomonas* (TCC, TCS), *Methylobacillus* (TCS) and *Stenotrophomonas* (TCS) were

among the most abundant (chemical degraded is shown in parenthesis). From all of the KEGG xenobiotic degrading pathways, genes from five pathways were the most frequently detected, including those associated with aminobenzoate, benzoate (most common), chlorocyclohexane/chlorobenzene, dioxin, and nitrotoluene biodegradation. Several phylotypes including *Bradyrhizobium*, *Mycobacterium, Rhodopseudomonas, Pseudomonas, Cupriavidus,* and *Streptomyces* were common genera associated with these pathways. Overall, the data suggest several phylotypes are likely involved in the biodegradation of these PPCPs, with *Pseudomonas* being a probable important genus for all three chemicals.

4.2. Introduction

The antiepileptic drug carbamazepine (5H-dibenzo [b, f] azepine-5-carboxamide) (CBZ) and the antimicrobials, triclocarban (3-(4-chlorophenyl)-1-(3, 4-dichlorophenyl) urea) (TCC) and triclosan (2, 4, 4'-trichloro-2'-hydroxydiphenyl ether) (TCS), are not fully removed during the wastewater treatment process (Cha and Cupples, 2009; Clara et al., 2005; Kandel et al., 2017; Miao and Metcalfe, 2003; Wu et al., 2010), and are released into agricultural soils via irrigation with wastewater effluents and biosolids application (Al-Rajab et al., 2015; Cha and Cupples, 2009; Grossberger et al., 2014b; Kwon et al., 2010; Monteiro and Boxall, 2009; Paz et al., 2016). The presence of CBZ, TCC, and TCS in agricultural soils is problematic due to their recalcitrance and the potential for translocation into plants. Several researchers have documented the recalcitrant nature of CBZ in soil following irrigation with wastewater effluent (Gibson et al., 2010; Grossberger et al., 2014a; Kinney et al., 2006), with half-lives ranging from 355-1,624 days (Dalkmann et al., 2014). CBZ concentrations in biosolids and soils have ranged from 5 ng g^{-1} to 258 ng g^{-1}(Ding et al., 2011; Gottschall et al., 2012; Morais et al., 2013; Radjenovic et al., 2009; Spongberg and Witter, 2008) and from 1.8 to 7.5 ng g^{-1} (Gibson et al., 2010; Vazquez-

Roig et al., 2010; Walker et al., 2012), respectively. A recent review placed CBZ on a list of chemicals with a high bioaccumulation factor in roots and in leaves/stems (Wu et al., 2015). TCC and TCS are also particularly common in biosolids and agricultural soils (Barber et al., 2006; Bendz et al., 2005; Bester, 2005; Boyd et al., 2004; Haggard et al., 2006; Halden and Paull, 2005; Heidler and Halden, 2007; Hua et al., 2005; Kanda et al., 2003; Kolpin et al., 2002; Loraine and Pettigrove, 2006; McAvoy et al., 2002; Morrall et al., 2004; Sabaliunas et al., 2003; Singer et al., 2002; Stackelberg et al., 2004; Thomas and Foster, 2005; Thompson et al., 2005; Waltman et al., 2006). TCC and TCS have been detected in biosolids at concentrations ranging from 2,900 to 51,000 ng g^{-1} (Cha and Cupples, 2009; Chu and Metcalfe, 2007; Gottschall et al., 2012; Wu et al., 2010) and from 90 to 11,550 ng g^{-1} (Cha and Cupples, 2009; Chu and Metcalfe, 2007; Ying and Kookana, 2007), respectively. In soils, TCC has been measured from between 1.2 and 200 ng g^{-1}(Cha and Cupples, 2009; Chen et al., 2011; Wu et al., 2010) whereas TCS concentrations varied from 0.16 to 3.1 ng g^{-1}(Cha and Cupples, 2009; Wu et al., 2010). TCC and TCS were also listed as having high bioaccumulation factors in roots compared to other chemicals (Wu et al., 2015). Further, TCS has a tendency to accumulation in leaves/stems (Wu et al., 2015). In light of these findings, an understanding of the removal of these chemicals from agricultural soils is warranted.

Biodegradation of CBZ, TCC and TCS by soil microorganisms is a plausible removal mechanism from agricultural soils. However, little is known about the dominant phylotypes and functional genes associated with their removal. Although CBZ, TCC and TCS degrading isolates have been obtained, it is generally unknown if these microorganisms are active and present in agricultural soils. Microorganisms associated with CBZ biodegradation include *Rhodococcus rhodochrous*, *Aspergillus niger* (Gauthier et al., 2010), *Starkeya* sp. C11, *Rhizobium* sp. C12,

Pseudomonas sp. CBZ-4 (Bessa et al., 2017; Li et al., 2013) and *Streptomyces* MIUG 4.89 (Popa et al., 2014). Bacteria associated with TCC biodegradation include *Alcaligenaceae* (Miller et al., 2008), *Sphingomonas* sp. strain YL-JM2C, *Ochrobactrum* sp. TCC-1 (Mulla et al., 2016a; Yun et al., 2017a), *Ochrobactrum* sp. TCC-2, *Pseudomonas fluorescens* MC46 and *Ochrobactrum* sp. MC22 (Sipahutar et al., 2018; Sipahutar and Vangnai, 2017; Yun et al., 2017b). TCS-degrading bacteria include *Methylobacillus* sp., *Sphingomonas* sp. strain YL-JM2C, *Sphingopyxis* strain KCY1 (Lee et al., 2012; Lolas et al., 2012; Mulla et al., 2016b), *Pseudomonas putida* TriRY, and *Alcaligenes xylosoxidans* subsp. *denitrificans* TR1 (Meade et al., 2001). Again, it is unclear if these microorganisms are actively involved in CBZ, TCC, and TCS degradation in agricultural soils at environmentally relevant concentrations.

One objective of the current study was to determine if specific phylotypes could be associated with CBZ, TCC and TCS degradation in four agricultural soils subjected to different cropping and agronomic management systems (Robertson and Hamilton, 2015). In contrast to isolation studies, which typically focus on higher concentrations of the targeted chemical (often ppm levels), the current study targeted lower (ppb) concentrations, closer to those found in the environment. A second objective was to compare the occurrence of genes associated with a group of KEGG (Kyoto Encyclopedia of Genes and Genomes Orthology, KEGG Orthology) (Kanehisa, 2002) xenobiotic degradation pathways between the samples amended with CBZ, TCC and TCS and the live controls (no chemical added) to determine if specific functional genes could be linked to biodegradation. A third objective was to compare the phylotypes and functional genes associated with this set of xenobiotic degrading pathways between all four soils to ascertain if the cropping regimes impacted the functional abilities of the four soil microbial communities.

Previously, 16S rRNA gene amplicon sequencing was used to obtain taxonomic profiles of soil microbial communities following exposure to CBZ and TCC (Illumina MiSeq) (Thelusmond et al., 2018; Thelusmond et al., 2016). Here, we expand on this research, using shotgun sequencing (Illumina HiSeq) to provide data on both the taxonomic and functional profiles of agricultural soil microbial communities following exposure to CBZ, TCC and TCS.

4.3. Methods

4.3.1. Chemicals and Materials

CBZ and TCC (purity >99%) were purchased from Sigma-Aldrich (Saint-Louis, MO, USA), and TCS (purity >99%) was obtained from Tokyo Chemical Industry (Portland, OR, USA). CBZ-d_{10} and TCS-d_3 (purity >99.4%) were from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada) and TCC-$^{13}C_6$ (purity ≥98%) was acquired from Cambridge Isotope Laboratories (Andover, MA, USA). HPLC grade acetonitrile and LC/MS grade methanol were from Fisher Scientific. QuEChERS (quick, easy, cheap, effective, rugged, and safe) extract pouches (6.0 g magnesium sulfate and 1.5 g sodium acetate) (Anastassiades et al., 2003) were from Agilent Technologies (Wilmington, DE, USA). Talcum powder (Medical grade) was purchased from Fisher Scientific (Dallas, TX, USA). Cleanert SAX (1000mg, 6 cc), Oasis HLB (200 mg, 6cc), and Strata™-X (200 mg, 6 cc) cartridges were from GS-Tek (Newark, DE, USA), Waters (Milford, MA, USA), and Phenomenex (Torrance, CA, USA). The PowerSoil DNA isolation kit was from MO BIO Laboratories (Carlsbad, CA, USA).

4.3.2. Soils and the Experimental Design

Batch microcosms spiked with CBZ, TCC or TCS were established with four soils (hereafter, soils 1, 2, 3, and 4) collected from 5 sampling stations in 6 replicate plots for Treatments 1, 2, 3 and 4 within the Michigan State University Main Cropping System

Experiment at Kellogg Biological Station Long-Term Ecological Research (KBS LTER) (42°24'N, 85°23'W). Treatments 1 and 2 receive conventional levels of chemical inputs. Treatment 1 is chisel plowed and Treatment 2 is under no-till management. Treatments 3 and 4 are biologically based low chemical input systems with a winter leguminous cover crop. Treatment 3 receives banded herbicide and starter nitrogen at planting, and Treatment 4 (certified organic) receives no chemical inputs nor compost or manure. Both Treatment 3 and Treatment 4 receive additional post-planting cultivation and Treatment 4 is rotary-hoed to control weeds. The physical and chemical characteristics of the soils were determined by A & L Great Lakes Laboratories, Inc. (Fort Wayne, IN) (Supplementary Table 4.1). For additional information see https://lter.kbs.msu.edu/research/site-description-and-maps/. The experimental design involved sacrificial sampling of soil microcosms for chemical analysis at days 2, 40 and 80 during CBZ or TCC degradation or days 2, 15, and 25 during TCS degradation. DNA was extracted (as described below) at day 15 (TCS) and day 80 (TCC and CBZ).

To initiate the experiment, triplicate serum bottles, containing 5 g soil, were amended with 50 ng g^{-1} of CBZ or TCS dissolved in water. TCC (50 ng g^{-1} soil) was introduced using talcum powder, as previously described (Thelusmond et al., 2018). The moisture contents of the soils were adjusted to 60% of water holding capacity. The live controls (also in triplicate), treated in the same manner, except no chemical was added, were used to determine the effect of each chemical on the soil metagenomes. Triplicate abiotic controls, obtained by autoclaving for three consecutive days, were also included. Each chemical and soil combination resulted in 27 microcosms (3 live microcosms, 3 control live microcosms, 3 abiotic controls, 3 time points for sacrificial sampling). Therefore, for each chemical, 108 microcosms (27 X 4 soils) were required to account for the 4 soils. Twenty-four DNA extracts (4 soils, 3 chemicals, 2 treatments) were

submitted for shotgun sequencing (as described below).

4.3.3. CBZ, TCC and TCS Extraction, Analysis and Solid Phase Extraction

CBZ, TCC and TCS were extracted and analyzed using a modified QuEChERS approach (Salvia et al., 2012; Thelusmond et al., 2018; Thelusmond et al., 2016). Soil matrices were spiked with internal standards (20 ng g^{-1}) before extraction. Briefly, 10 mL of DI water plus 15 mL of acetonitrile were added to the soil samples in 50-mL centrifuge tubes followed by vortex-mixing for 2 min; then slowly and continuously, the QuEChERS buffer (6 g of magnesium sulfate and 1.5 g of sodium acetate) was added while vortexing at a low speed (Braganca et al., 2012). The tubes were shaken by hand for 30 sec and then vortexed at a high speed for 30 sec. The tubes were then placed on a shaker (Lab-Line, Lab-Line Instruments, Inc. Melrose, IL) at 750 rpm for 3 min before centrifugation at 3000 g (2 min). Then, 10 mL of the acetonitrile supernatant was placed in a 12-mL glass tube followed by evaporation to dryness through a gentle stream of nitrogen at 40 ^{o}C and resuspension in 60 mL of methanol-water (3:97, v/v). The reconstituted soil extracts were subjected to solid phase extraction prior to their analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). The solid phase extraction (SPE) technique was previously described (Salvia et al., 2012; Thelusmond et al., 2018; Thelusmond et al., 2016; Vazquez-Roig et al., 2010). The collected eluate from SPE was evaporated to dryness under a stream of nitrogen at 40^{o}C and reconstituted with 1 mL of methanol before transferring to 1.5 mL HPLC vials and storing at -15^{o}C until LC-MS/MS analysis. Before initiating the batch experiments, the percent recovery for each chemical was determined for each soil. Triplicates of soil samples were spiked overnight with 50 ng g^{-1} of each chemical which was extracted the next day using the extraction procedure described above. The soil extracts were purified using SPE and then analyzed by LC-MS/MS as described above.

4.3.4. LC-ESI-MS/MS

CBZ, TCC and TCS analysis methods were previously developed (Cha and Cupples, 2009; Cha and Cupples, 2010; Thelusmond et al., 2018; Thelusmond et al., 2016). For CBZ, mass spectrometry was performed using an LC-MS/MS system with a Shimadzu HPLC coupled with an API SCIEX 3200 triple quadrupole mass spectrometer equipped with TurboIonSpray ion source. CBZ and CBZ-d_{10} were ionized in positive mode with ion spray voltage and temperature set to 5000 V and 750 ^{o}C, respectively. The curtain gas pressure was set to 10 psi, whereas the collision gas pressure and the ion source gas pressure were set to 9 psi and 30 psi, respectively. The MS/MS parameters were previously optimized for both CBZ and CBZ-d_1 (Thelusmond et al., 2018). The HPLC system was comprised of two LC-20AD pumps, an SIL-20A autosampler and a DGU-20A degasser with a CBM-20A controller. A Gemini C18 column (50 x 2.00 mm, 5µm, Phenomenex) was used for the LC separation. The mobile phases consisted of 0.1% formic acid in MilliQ water (A) and acetonitrile (B). The flow rate was 0.3 mL/min and the injection volume was 10 µL. For TCC and TCS, LC was conducted with a Waters ACQUITY Ultra Performance LC (UPLC) system, consisting of an auto sampler and a binary pump. TCC and TCS were separated using a Waters ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 x 50 mm) at 50 ^{o}C. Mobile phase A (0.1% formic acid in MilliQ water) and mobile phase B (acetonitrile) were used to produce a binary elution gradient with a flow rate of 0.30 mL/min for TCC and TCS. The separation of TCC and TCS was achieved with the following linear mobile phase gradient program: at 0 min, A/B=99:1 (v/v); at 0.5 min, A/B=99:1 (v/v); at 2 min, A/B= 30:70 (v/v); at 3 min, A/B= 1:99 (v/v); at 4 min, A/B= 1:99 (v/v); at 4.01 min, A/B= 99:1 (v/v); at 5 min, A/B=99:1 (v/v). Each ionization method was optimized independently using separate source-tuning parameters. Ionization source was electrospray ionization (ESI). MASSLYNX

version 4.1 software was used to control the mass spectrometric conditions. The optimized tune page settings parameters have been summarized (Supplementary Table 4.2).

4.3.5. DNA Extraction, Shotgun HiSeq Illumina sequencing and MG-RAST analysis

DNA was extracted using the Power Soil DNA extraction kit (Mo Bio Laboratories, Inc. Carlsbad, CA, A Qiagen Company) following the manufacturer's instructions. Shotgun sequencing was performed using the Illumina HiSeq 4000 (2 X 150 bp) at the Research Technology Support Facility (RTSF) at Michigan State University on 24 DNA extracts (4 soils, 3 chemicals, samples and live controls). The shotgun sequences were analyzed with MG-RAST (Meyer et al., 2008) version 4.0.2. (Supplementary Table 4.3). The processing pipeline includes SolexaQA (Cox et al., 2010) to trim low-quality regions from FASTQ data and dereplication to remove artificial duplicate reads. Gene calling was completed using FragGeneScan (Rho et al., 2010). The MG-RAST analysis included the RefSeq database (Pruitt et al., 2005) and the KEGG database (Kanehisa, 2002). The sequencing data is publically available on MG-RAST.

4.3.6. Statistical analysis of the phylogenetic and functional data using STAMP

Statistical Analyses of Metagenomic Profiles (STAMP) software version 2.1.3. (Parks et al., 2014) was used to analyze the metagenomic data from the MG-RAST analysis. The STAMP analysis included generating heatmaps for the 25 most abundant phylotypes in each soil. Also, principal component analysis was performed separately for each chemical using both the CBZ, TCC or TCS amended soils and the live controls. Extended error bars were generated to illustrate which phylotypes were statistically different (Welch's two sided t-test, $p<0.05$) between the CBZ, TCC and TCS amended soils compared to the live controls. Heatmaps were also generated for the 25 most abundant phylotypes associated with five xenobiotic degrading KEGG pathways (aminobenzoate, benzoate, chlorocyclohexane and chlorobenzene, dioxin and nitrotoluene

degradation). These were selected because they were the five most dominant KEGG xenobiotic degrading pathways from the sequencing data. Extended error bars were also generated to illustrate which genes in these xenobiotic degrading pathways were statistically different between the samples and live controls for each chemical. Similarly, extended error bars were generated to compare each soil individually to the other three soils for the genes associated with xenobiotic degradation. Finally, heatmaps were created to illustrate the most abundant genes associated with each of the five xenobiotic degrading pathways.

4.4. Results

4.4.1. Extraction Recovery and Degradation Data

Average percent recoveries of the chemicals from the four soils ranged between 80 ± 2% and 96 ± 7%, 93 ± 3% and 100 ± 14%, and 87 ± 3% and 97 ± 12% for CBZ, TCC and TCS, respectively (Supplementary Table 4.4). Figure 4.1 illustrates CBZ, TCC, and TCS concentrations in the four soils for the live microcosms and abiotic controls with respect to time. CBZ exhibited low removal (<50%) in all four soils over 80 days. For CBZ, for soils 1 and 2, statistically significant differences (t-test, $p<0.05$) were found between the samples and controls only for day 80 (when DNA was extracted from all four soils). In soil 3, CBZ concentrations were statistically significantly different between the samples and controls for both days 40 and 80. Whereas in soil 4, CBZ concentrations were significantly different between the controls and samples for all three time points. Likewise, TCC dissipation in soils was moderate with <50% removal over 80 days. For soils 1, 2 and 3, significant differences were noted in TCC concentrations between the samples and controls on both days 40 and 80. For soil 4, the TCC concentrations were only significantly different between the samples and controls on day 80 (when DNA was extracted from all four soils). In contrast to CBZ and TCC, TCS rapidly

dissipated in all four soils (Figure 4.1C). Significant differences (t-test, $p<0.05$) in TCS concentrations were found between the samples and controls on all days. On day 25, a reduction in TCS concentration of > 80% was recorded for the live samples, with limited reductions in the controls. Nucleic acids were extracted on day 15 for the TCS amended samples and live controls.

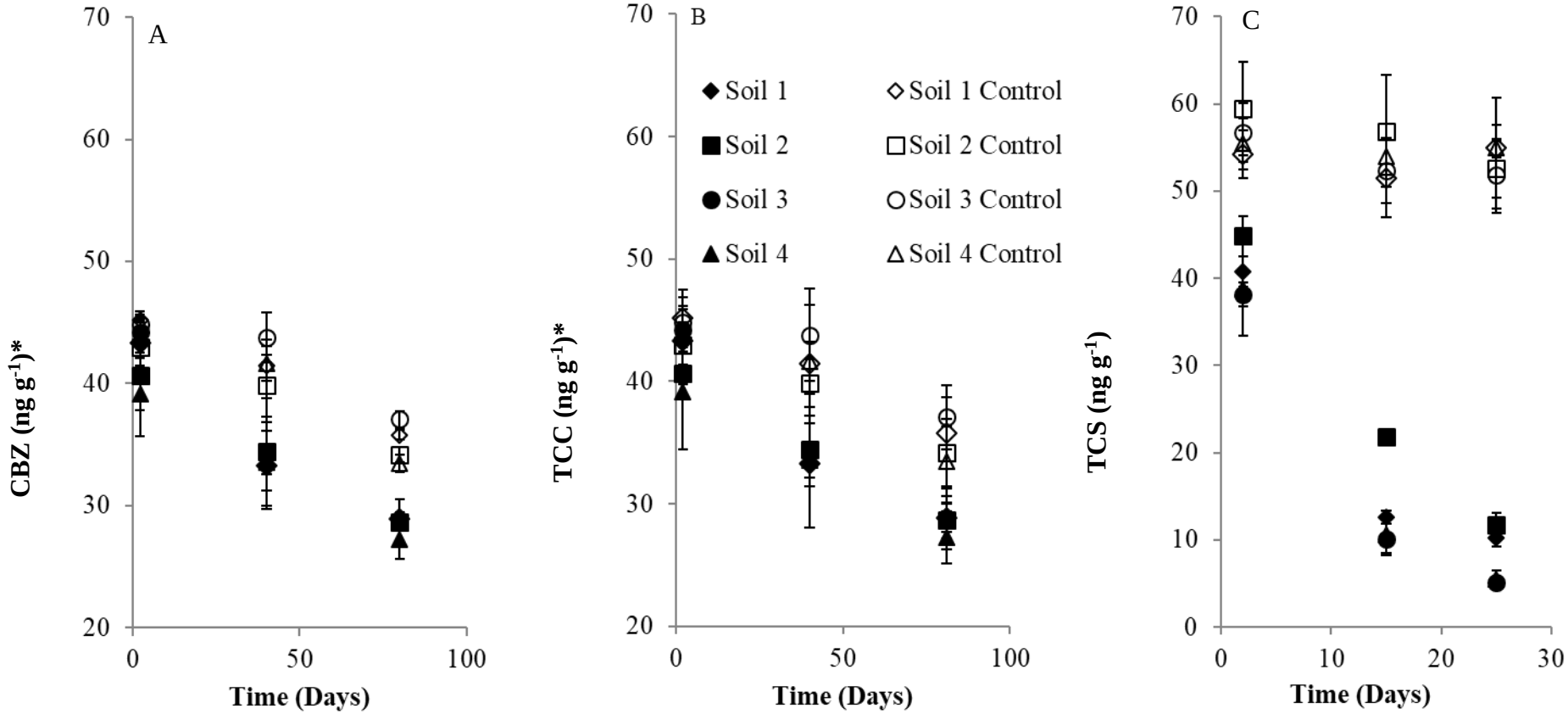


Figure 4.1 Average CBZ, TCC or TCS remaining (ng g^{-1}) under aerobic conditions in four soils in the live sample microcosms compared to abiotic controls. The bars represent standard deviations from three microcosms. *non-zero axis. Soils 1 and 2 were statistically significantly different from controls only on day 80, soil 3 was significantly different from controls on days 40 and 80, and soil 4 was significantly different from controls on all time points (A). All soils were statistically significantly different from the controls on days 40 and 80 (except soil 4, only significantly different on day 80) (B). All soils were statistically significantly different from the controls on all days (C).

4.4.2. Microbial Community Analysis

The 25 most abundant phylotypes for each soil in the CBZ, TCC (both on day 80) and TCS (day 15) amended samples and live controls (no chemical added) are shown (Figure 4.2). Six phylotypes (*Burkholderia, Streptomyces, unclassified* derived from *Verrucomicrobia subdivision 3, Gemmatimonas, Candidatus Solibacter*, and *Bradyrhizobium*) were dominant in all four soils in the CBZ, TCC or TCS amended samples as well as the live controls. Overall, *Burkholderia* and *Streptomyces* were the most abundant phylotypes in the samples and controls in all four soils. Other less abundant microorganisms were also present in all four soils e.g. *Rhodopseudomonas*, *Mycobacterium*, *Chthonibacter* and *Sorangium*. Differences between the soils include a higher abundance of *Xanthomonas* in four of the DNA extracts from soil 1. Also, *Mycobacterium* appeared more abundant in soil 3 compared to the other soils. Overall, although the soils were exposed to different cropping treatments, this appeared to have a limited effect on the identity of the most abundant phylotypes. No clear trends were noted for the effect of each chemical on these 25 most abundant phylotypes for each soil (data not shown).

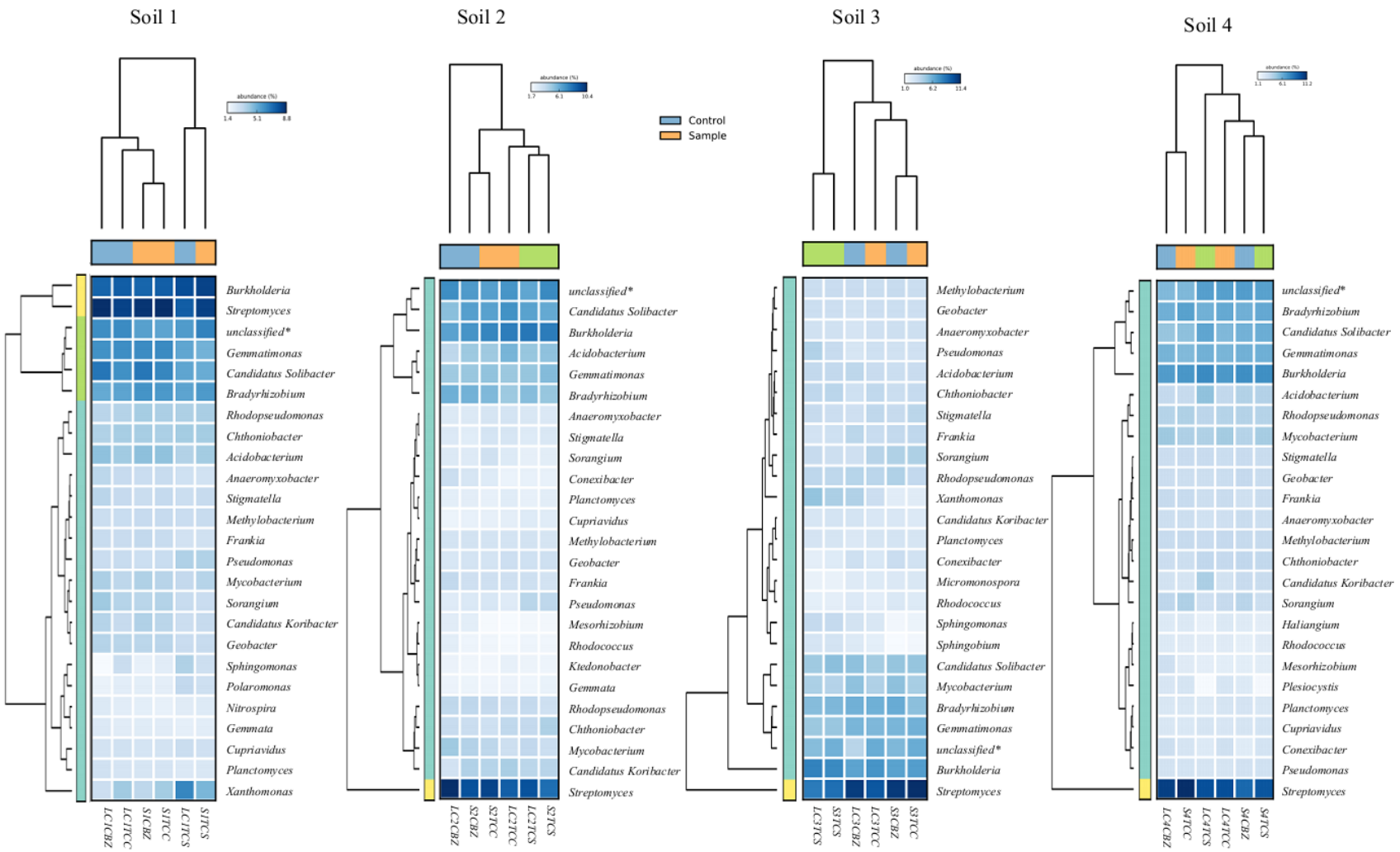


Figure 4.2 Heatmaps of the 25 most abundant phylotypes at day 15 in soils 1, 2, 3 and 4 from the TCS amended samples and controls (no TCS) and at day 80 from the CBZ or TCC amended samples and controls *derived from *Verrucomicrobia subdivision 3*

The principal component analysis (PCA) indicated the CBZ amended samples and no CBZ amended controls clustered closer together for soil 1 compared to soils 2, 3 or 4, suggesting the presence of CBZ had a greater impact on the microbial communities of soils 2, 3 and 4 (Figure 4.3A). The PCA for TCC illustrated closer clustering between the samples and controls for soils 1 and 4 compared to soils 2 and 3 (Figure 4.3B). For TCS, all four soils clustered separately, with soils 3 and 4 illustrating the closest clustering between the samples and controls (Figure 4.3C). One common trend being that soil 4 exhibited the least impact between the TCC or TCS amended samples and the controls. In contrast, soil 2 exhibited some of the larger separations between the controls and samples, perhaps suggesting the microbial community from this soil is more sensitive to exposure to these chemicals.

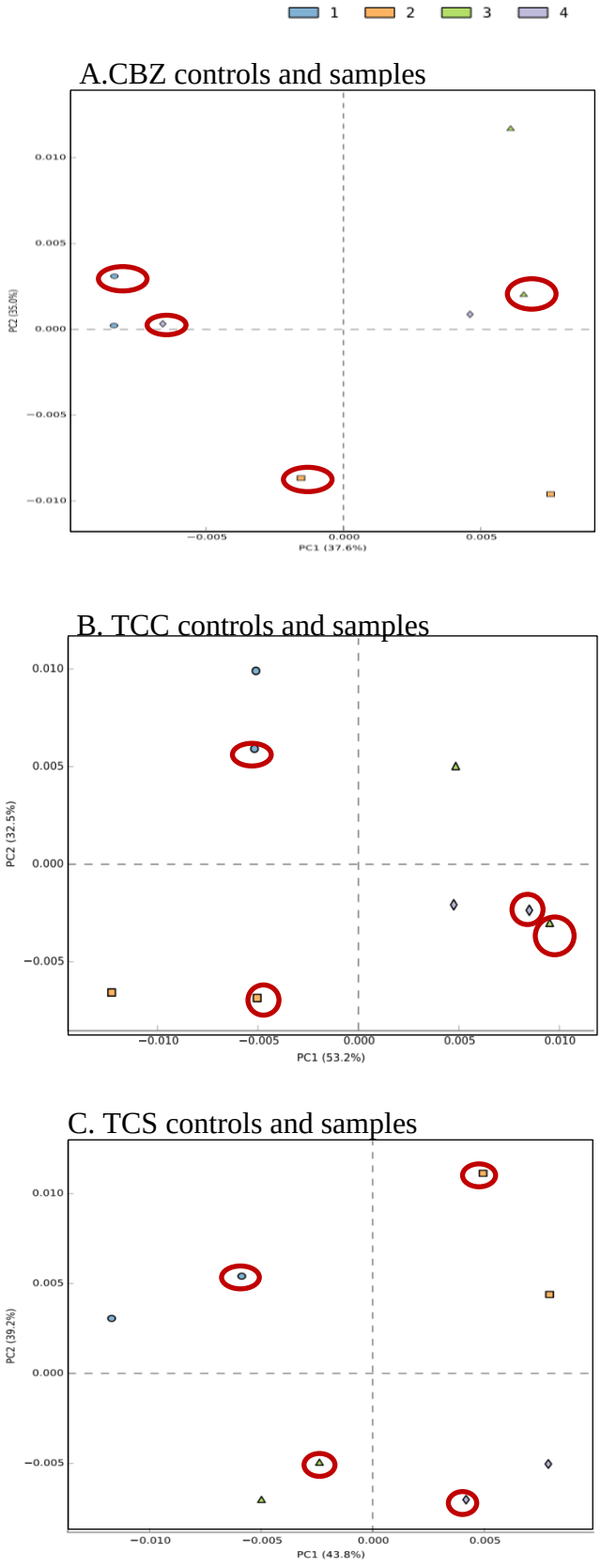


Figure 4.3 Principal component analysis of CBZ amended samples and no CBZ controls (A), TCC amended samples and no TCC controls (B), TCS amended samples and no TCS controls (C) for soils 1, 2, 3, and 4. Only day 80 for CBZ and TCC and day 15 for TCS were considered in the analysis. The samples are circled in red.

4.4.3. Carbamazepine, Triclocarban, and Triclosan Enriched Phylotypes

The data from all four soils were combined to determine which microorganisms were impacted by the presence of each chemical. The phylotypes present at significantly different levels between the live controls and the samples are shown (Figure 4.4). The complete classification of each phylotype has also been provided (Supplementary Tables 4.5-4.7). For each chemical, at least three phylotypes were enriched in the samples compared to the live controls or vice versa. For CBZ, three microorganisms (*Cytophaga, Maricaulis,* and *Sulfurihydrogenibium*) from three different phyla (*Bacteroidetes, Proteobacteria* and *Aquificae*) illustrated the largest positive impact from the presence of this chemical, with the largest negative impact of CBZ being on the genus *Pantoea* (*Proteobacteria*) (Figure 4.4A). For TCC, the most dominant trend was the negative impact of this chemical on phylotypes (*Bordetella, Herbaspirillum* and *Oxalobacter*) from the order *Burkholderiales* (phylum *Proteobacteria*). In contrast, the most dominant trend for TCS was the positive impact (although at a lower level) on phylotypes from a number of phyla. Overall, the data indicate minor common impacts of these chemicals when the four soil communities are considered together.

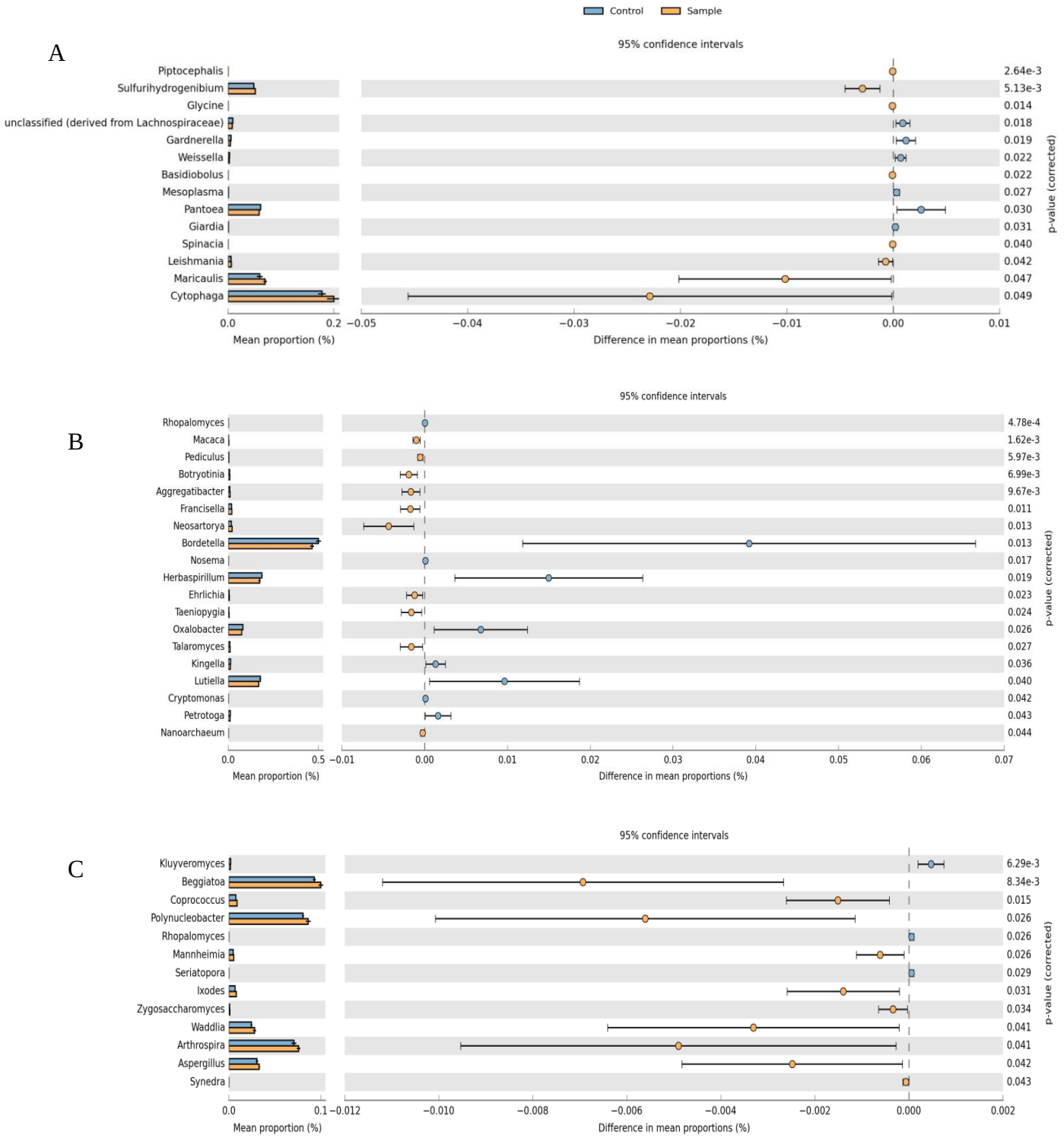


Figure 4.4 Phylotypes more abundant in the CBZ (A), TCC (B) and TCS (C) amended soils compared to the controls. The error bars on the left side of the dotted line indicate the phylotypes enriched in the samples whereas the error bars on the right side of the dotted line indicate the phylotypes enriched in the controls. The data from all four soils were combined together for this analysis.

The occurrence of previously reported CBZ, TCC and TCS degrading bacteria (at the genus level) was determined in all four soils (Table 4.1). The genera of all five CBZ degrading isolates were found in the current study and the relative abundance of each was similar between the samples and controls. The genera *Streptomyces* and *Pseudomonas* were the most abundant. Similarly, for TCC, at the genus level, the genera associated with five previous TCC degrading phylotypes were present in the soil samples and again *Pseudomonas* was the most abundant. For TCS, the genera of five TCS degrading bacteria were found in the soil, however, four were absent. From the five that were present, *Pseudomonas, Sphingomonas*, and *Methylobacillus* illustrated the highest abundance levels. Unfortunately, the MG-RAST data classifies reliably only to the genus level, therefore, it was not possible to ascertain if particular species or strains were present.

Table 4. 1 Organisms associated with CBZ, TCC, and TCS biodegradation in previous studies and in this study. The % relative abundance is separated by semi column for soils 1, 2, 3, and 4 in order for samples and controls.

Chemical	**Isolate in previous studies**	**Isolation sources**	**Similar genera in the current study**	**Genera % abundance in samples**	**Genera % abundance in controls**	**References**
CBZ	*Rhodococcus rhodochrous*	ATCC	*Rhodococcus*	0.64;0.78;0.88;0.65	0.63;0.97;0.88; 0.82	(Gauthier et al., 2010)
	Starkeya sp. C11	Activated Sludge	*Starkeya*	0.27;0.29;0.27;0.28	0.24;0.29;0.26;0.28	(Bessa et al., 2017)
	Pseudomonas sp. CBZ-4	Activated Sludge	*Pseudomonas*	1.07;1.081.04;0.99	1.09;1.04;0.96;1.02	(Li et al., 2013)
	Streptomyces MIUG 4.89	Soil	*Streptomyces*	3.03;3.4;3.8; 3.05	3.09; 4.00;4.00; 3.70	(Popa et al., 2014)
	Rhizobium sp. C12	Activated Sludge	*Rhizobium*	0.68; 0.69;0.72;0.71	0.65; 0.66; 0.73;0.72	(Bessa et al., 2017)
TCC	unclassified *Alcaligenaceae*	Activated Sludge	*Bordetella*	0.56;0.52; 0.56;0.55	0.51;0.50;0.51;0.50	(Miller et al., 2008)
	Sphingomonas sp strain YL-JM2C	Activated Sludge	*Sphingomonas*	0.80;0.55;0.56; 0.60	1.09;0.59;0.90;0.54	(Mulla et al., 2016a)
	Ochrobactrum sp. TCC-1	Activated Sludge	*Ochrobactrum*	0.12;0.12;0.11;0.12	0.11;0.11;0.12;0.12	(Yun et al., 2017a)
	Ochrobactrum sp. TCC-2	River Sediment	*Ochrobactrum*	0.12;0.12;0.11;0.12	0.11;0.11;0.12;0.12	(Yun et al., 2017b)
	Ochrobactrum sp. MC22	Soil	*Ochrobactrum*	0.12;0.12;0.11;0.12	0.11;0.11;0.12;0.12	(Sipahutar and Vangnai, 2017)
	Pseudomonas fluorescens MC46	Soil	*Pseudomonas*	1.14;1.14;1.08;0.99	1.13;1.04;1.09;0.98	(Sipahutar et al., 2018)
TCS	*Pseudomonas putida* TriRY	Compost/ Water/Soil	*Pseudomonas*	1.31;1.47;1.21;1.01	1.30;1.52;1.50;0.95	(Meade et al., 2001)
	Alcaligenes xylosoxidans subsp. *denitrificans* TR1	Compost/ Water/Soil	*Bordetella*	0.56;0.53;0.55;0.54	0.57;0.53;0.56;0.53	(Meade et al., 2001)
	Sphingomonas sp. strain YL-JM2C	Activated Sludge	*Sphingomonas*	1.05;0.73;1.25;1.10	1.29;0.83;1.23;1.14	(Hay et al., 2001; Kim et al., 2011; Mulla et al., 2016b)
	Methylobacillus sp.	Activated Sludge	*Methylobacillus*	1.19;1.09;0.53;0.40	1.19;0.70;0.40;0.40	(Lolas et al., 2012)
	Novosphingobium sp. TrD22	Activated Sludge	Not found			(Zhou et al., 2013)
	Sphingopyxis strain KCY1	Activated Sludge	Not found			(Lee et al., 2012)
	Alicycliphilus	Activated Sludge	*Alicycliphilus*	0.16;0.15;0.16;0.15	0.18;0.14;0.16;0.15	(Lee et al., 2014)
	Stenotrophomonas	Activated Sludge	*Stenotrophomonas*	0.68;0.51;0.62;0.72	0.88;0.61;0.73;0.71	(Lee et al., 2014)
	Dyella sp.	Activated Sludge	Not found			(Wang et al., 2018)
	Nitrosomonas europaea	Activated Sludge	Not found			(Roh et al., 2009)

4.4.4. Phylotypes Associated with Xenobiotic Degrading Pathways

The most abundant phylotypes associated with five xenobiotic degrading KEGG pathways (aminobenzoate, benzoate, chlorocyclohexane and chlorobenzene, dioxin and nitrotoluene degradation) were determined for each soil (Figures 4.5-4.9). These pathways were selected because of their dominance in the sequencing data compared to the 16 other KEGG xenobiotic degrading pathways (data not shown). For all soils, *Bradyrhizobium* was the most abundant phylotype associated with pathways encoding for aminobenzoate biodegradation for CBZ, TCC, or TCS amended samples and controls, with the exception of soil 4 with or without TCS (Figure 4.5). Other abundant phylotypes associated with pathways encoding for aminobenzoate biodegradation included *Burkholderia, Cupriavidus, Ralstonia* (soils 1, 2, 3), *Sorangium* (soil 2), and *Polaromonas* (soil 2). In soil 4, the diversity of phylotypes associated with aminobenzoate degradation was more limited than the other soils.

For pathways encoding for benzoate degradation, the genus *Rhodopseudomonas* was the most abundant in soils 1, 2, and 4 in both CBZ, TCC, and TCS amended samples and controls, whereas *Pseudomonas* was the most abundant in soil 3 for all the chemicals (Figure 4.6). Considering all soils and chemicals, the most abundant phylotypes encoding for the benzoate degradation were *Polaromonas, Cupriavidus, Bradyrhizobium, Burkholderia, Pseudomonas* and *Ralstonia* (Figure 4.6). In contrast, only 3 phylotypes (*Bradyrhizobium, Candidatus Solibacter,* and *Burkholderia*) were associated with pathways encoding for chlorocyclohexane and chlorobenzene biodegradation for all soils and chemicals (Figure 4.7). For the phylotypes associated with pathways encoding for dioxin biodegradation, overall *Bradyrhizobium* was the most abundant across all four soils. However, variations in abundance levels for this genus were noted between treatments. For example, in soil 4, this phylotype was more dominant for the TCC

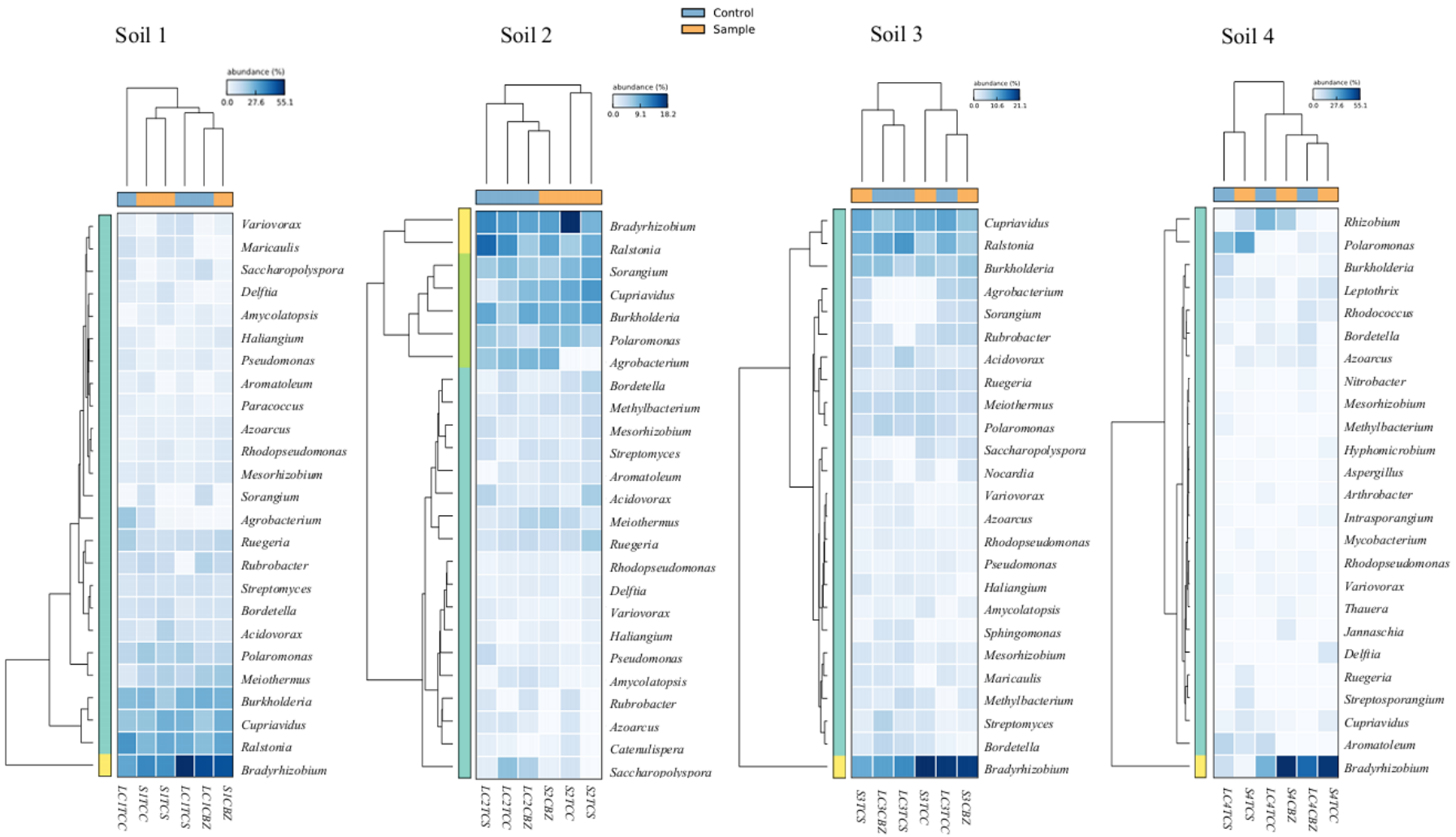


Figure 4.5 Heatmaps of the 25 most abundant phylotypes associated with pathways encoding for aminobenzoate biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for soils 1, 2, 3, and 4 at day 80 (CBZ or TCC) and day 15 (TCS).

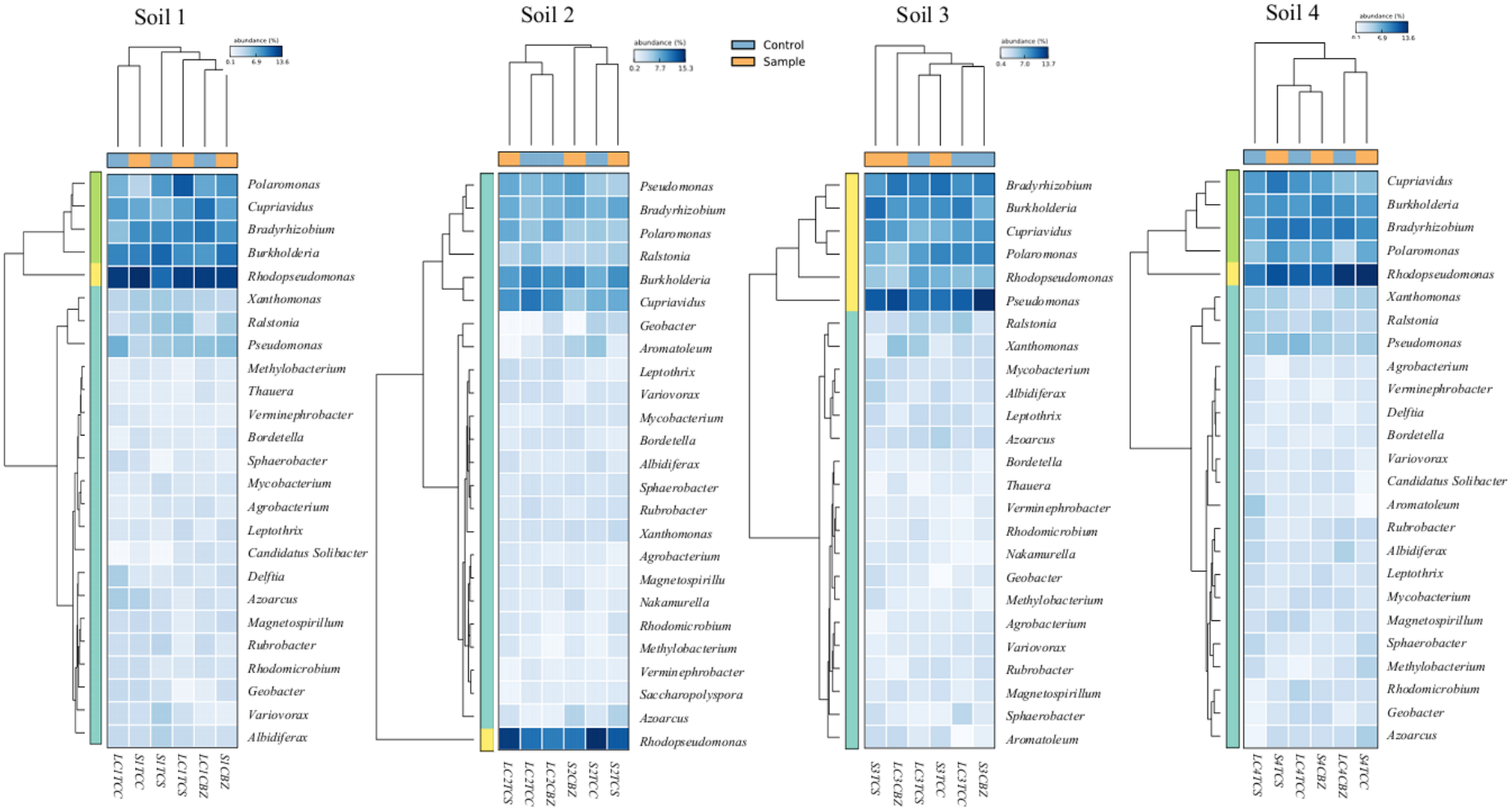


Figure 4.6 Heatmaps of the 25 most abundant phylotypes associated with pathways encoding for benzoate biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for soils 1, 2, 3, and 4 at day 80 (CBZ or TCC) and day 15 (TCS).

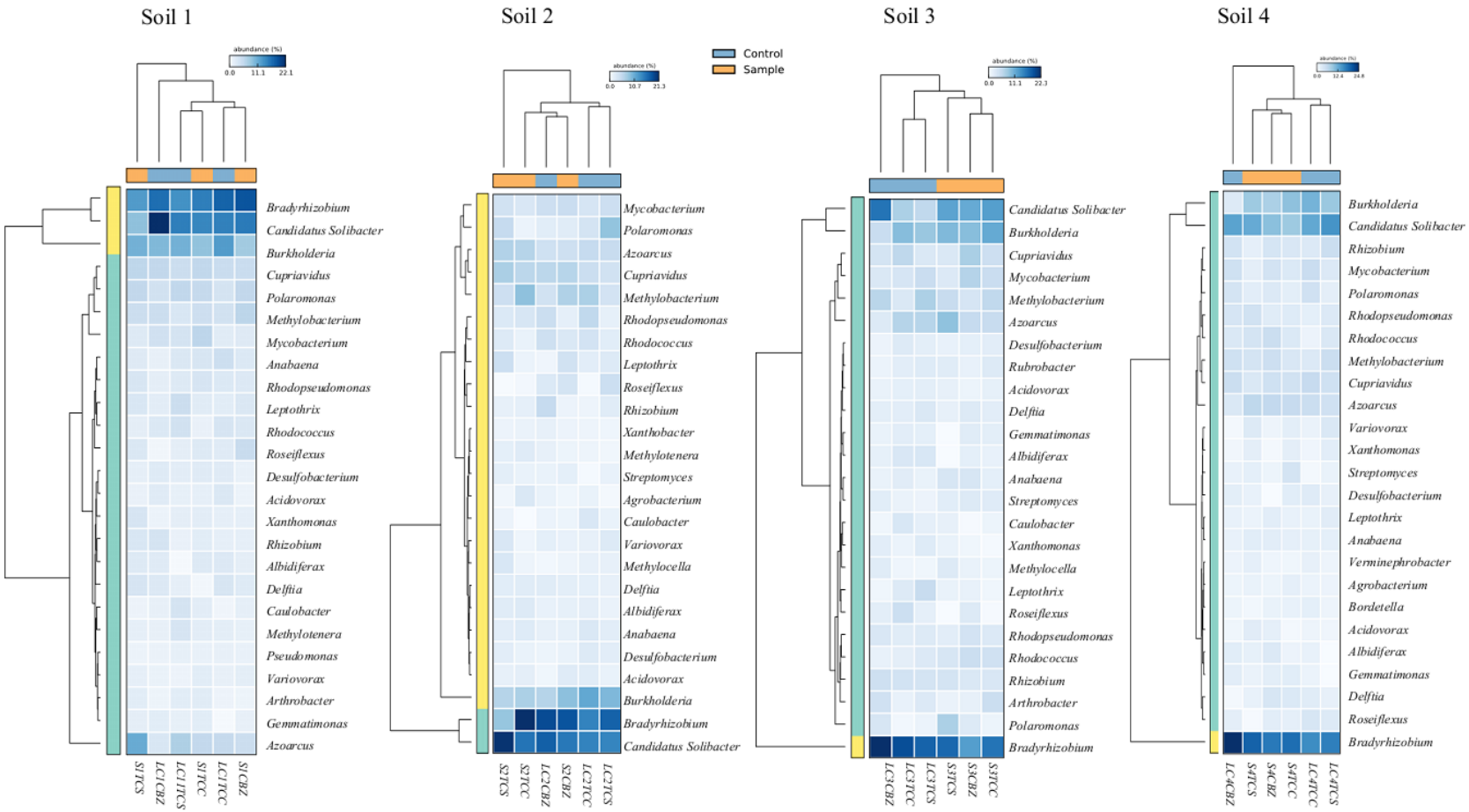


Figure 4.7 Heatmaps of the 25 most abundant phylotypes associate chlorocyclohexane and chlorobenzene biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for soils 1, 2, 3, and 4 at day 80 (CBZ or TCC) and day 15 (TCS).

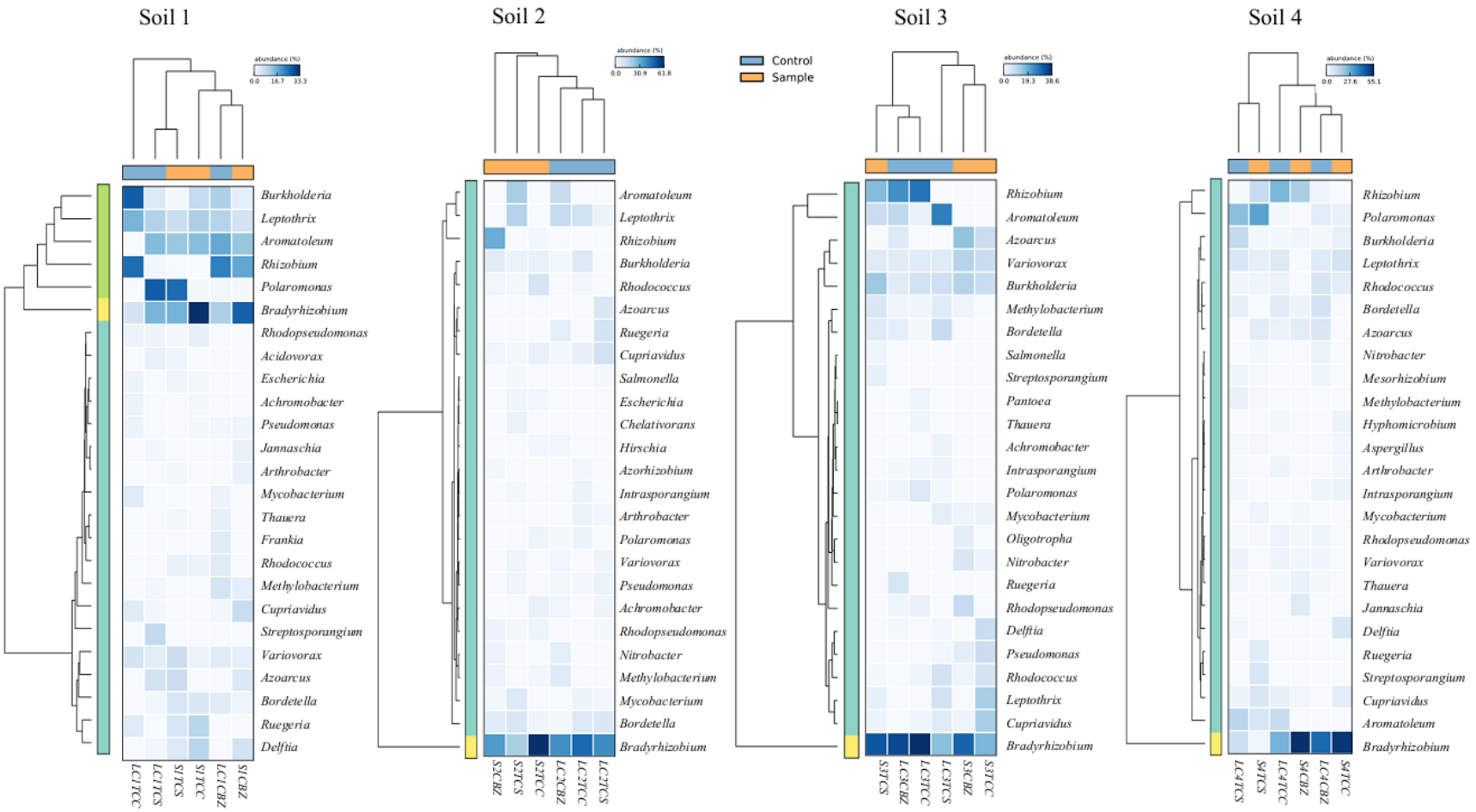


Figure 4.8 Heatmaps of the 25 most abundant phylotypes associated with pathways encoding for dioxin biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for soils 1, 2, 3, and 4 at day 80 (CBZ or TCC) and day 15 (TCS).

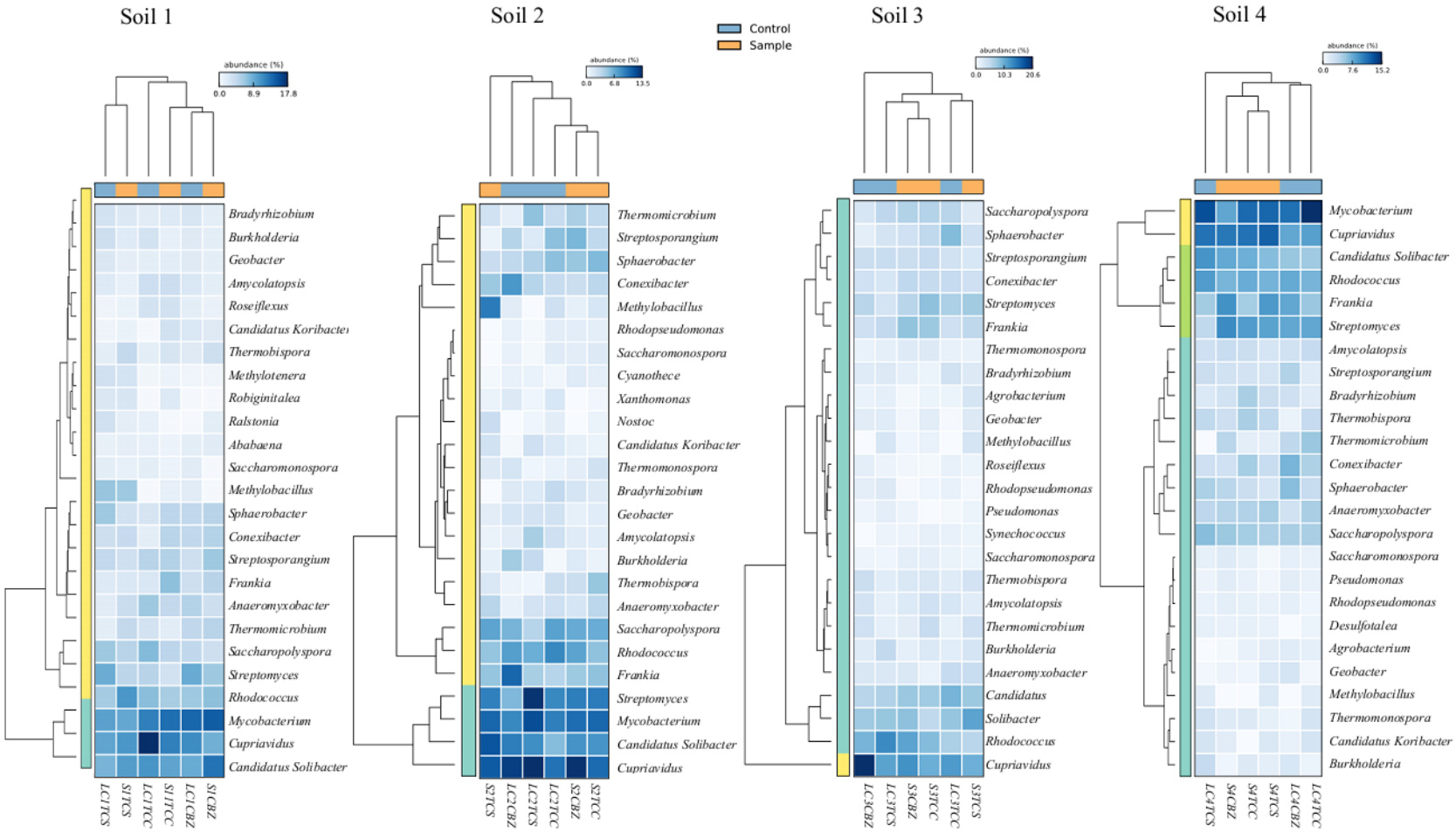


Figure 4.9 Heatmaps of the 25 most abundant phylotypes associated with pathways encoding for nitrotoluene biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for soils 1, 2, 3, and 4 at day 80 (CBZ or TCC) and day 15 (TCS).

and CBZ amended samples and controls compared to the TCS amended samples and controls. Other abundant phylotypes with genes associated with dioxin biodegradation included *Polaromonas* (soils 1 and 4), *Aromatoleum* (soils 1, 2, 3 and 4) and *Rhizobium* (soils 1, 2, 3, 4). A number of phylotypes were commonly associated pathways encoding for nitrotoluene biodegradation in all four soils (*Cupriavidus, Candidatus Solibacter, Mycobacterium, Streptomyces, Rhococcus, Methylobacillus,* and *Frankia*) (Figure 4.9). In soils 1 and 2, *Cupriavidus, Candidatus Solibacter* and *Mycobacterium* were dominant. In soil 3, *Cupriavidus, Candidatus Solibacter* and *Rhodococcus* were dominant. Whereas in soil 4, *Cupriavidus* and *Mycobacterium* were the most abundant phylotypes associated with nitrotoluene degradation.

4.4.5. Genes Associated with Different Metabolic Pathways

The heatmaps of the genes associated with pathways encoding for aminobenzoate, benzoate, chlorocyclohexane and chlorobenzene, dioxin and nitrotoluene degradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for all four soils combined at day 80 (CBZ or TCC) and day 15 (TCS) are also presented (Supplementary Figures 4.1-4.5). Twelve genes were associated with the pathways encoding for aminobenzoate biodegradation, with the most abundant being the gene encoding for a monooxygenase (anthraniloyl-CoA monooxygenase [EC: 1.14.13.40]) (Supplementary Figure 4.1). In contrast, forty-two genes were associated with benzoate degradation pathways, with six being more abundant than the rest (badA; benzoate CoA ligase[EC:6.2.1.25], pcaH; protocatechuate 3,4-dioxygenase beta subunit, pcaC 4-carboxymuconolactone decarboxylase [EC:4.1], ligJ; 4-oxalmesaconate hydratase [EC:4.2.1.83], pcaB 3-carboxy-cis,cis-muconate cycloisomerase, and pobA p-hydroxybenzoate 3-monooxygenase [EC:1.14.13.2]) (Supplementary Figure 4.2). Fifteen genes were associated with chlorocyclohexane and

chlorobenzene metabolism, with carboxymethylenebutenolidase [EC 3.1.1.45] being the most dominant (Supplementary Figure 4.3). Only three genes were associated with dioxin biodegradation, with the most abundant being salicylate hydroxylase [EC1.14.13.1] (Supplementary Figure 4.4). Finally, six genes were identified as part of nitrotoluene degradation pathways with the hydrogenase large subunit [E1.12.99.6L] and hydrogenase small subunit [E1.12.99.6S] being the most important (Supplementary Figure 4.5). The abundance of the genes associated with these xenobiotic degrading pathways were examined to determine if significant differences existed between the CBZ, TCC or TCS amended samples and controls (Figure 4.10). For each chemical, only two or three genes were significantly different between the samples and live controls. For CBZ, two genes (nemA; N-ethylmaleimide reductase [EC: 1. --] and hbaB, hcrC; 4-hydroxybenzoyl-CoA reductase subunit) were more abundant in the controls, and these were associated with benzoate and nitrotoluene pathways (Figure 4.10A). For TCC, two genes (had; 6-hydroxyclohex-1-ene-1-carboxyl-CoA dehydratase and bphA; biphenyl 2, 3-dioxygenase subunit alpha [EC: 1.14.12.18] belonging to benzoate and dioxin degradation pathways were more abundant in the samples compared to the controls, whereas one gene (badF benzoyl-coA reductase subunit [1.3.7.8]), associated with benzoate degradation pathway, was more abundant in the controls (Figure 4.10B). For TCS, one was more abundant in the samples (pobA; p-hydroxybenzoate 3-monoxygenase [EC: 1.14.1…]) and one was more abundant in the control (catB; muconate cycloisomerase [EC: 5.5.1.1]) with pathways belonging to benzoate and chlorocyclohexane and chlorobenzene degradation, respectively (Figure 4.10C). Overall, it appears that the presence of each chemical resulted in a limited impact on these xenobiotic degrading pathways.

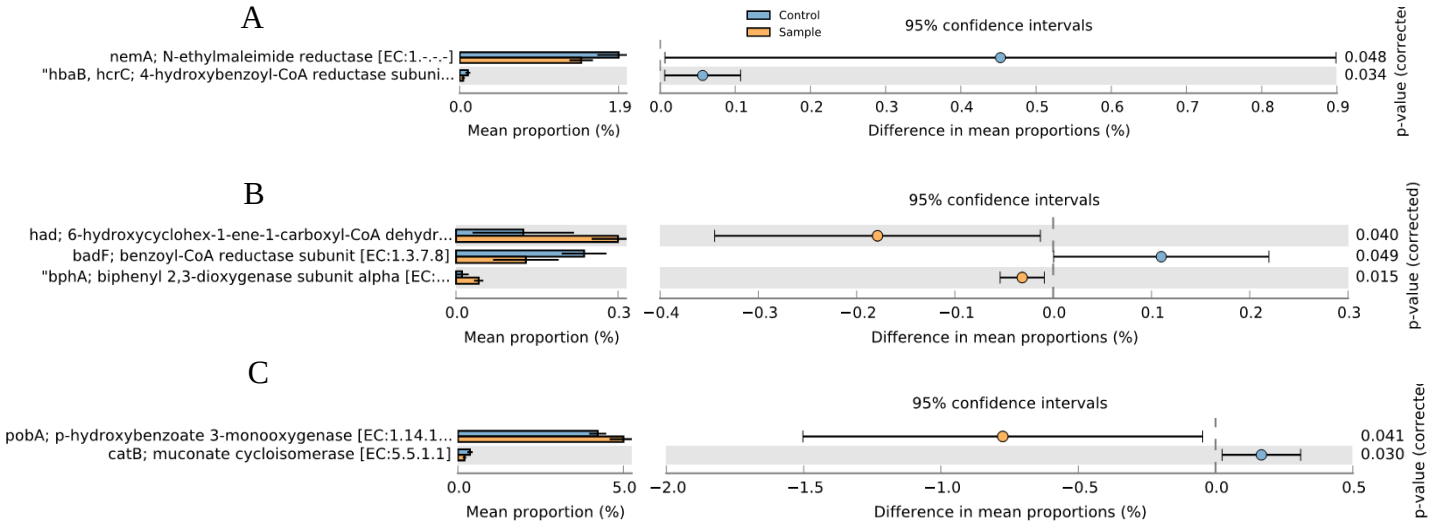
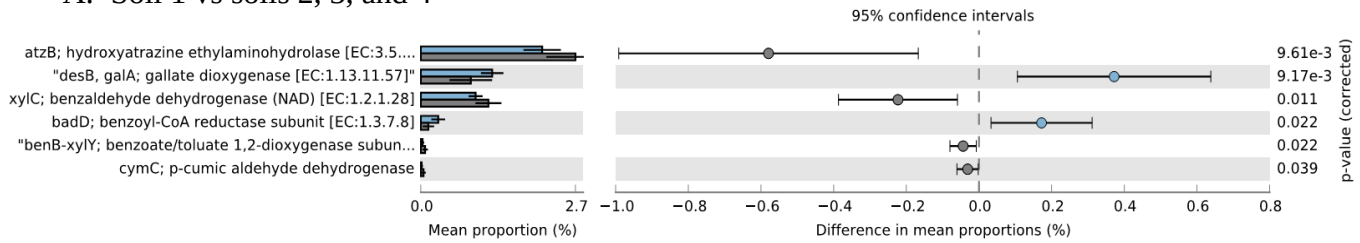


Figure 4.10 Genes significantly different in the CBZ (A), TCC (B) and TCS (C) amended soils (all soils combined) compared to the controls. Values on the left side of the dotted line indicate the genes significantly different in the samples whereas values on the right side indicate the genes significantly different in the controls.

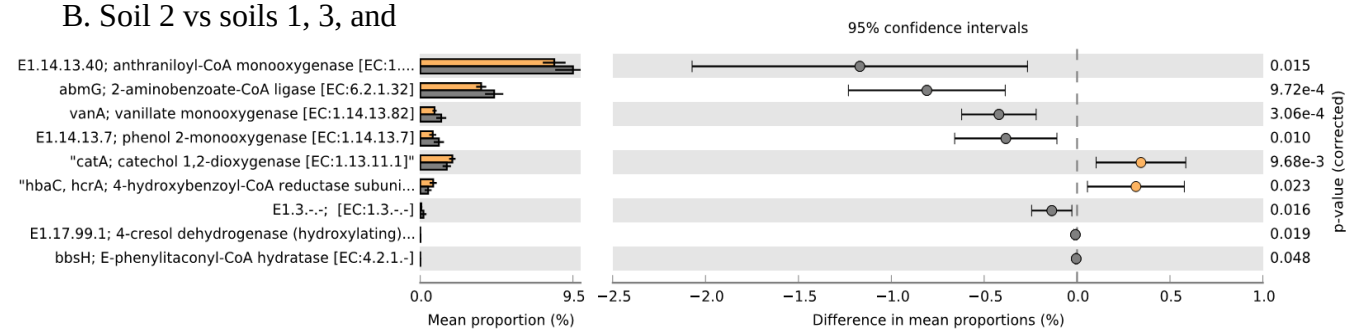
To determine if there were differences in the genes associated with xenobiotic degradation between soils, each soil was compared individually to the other three soils (Figure 4.11). Each soil contained between two and three genes that were more abundant compared to the other soils. The more abundance genes for soil 1 were desB, galA; gallate dioxygenase [EC: 1.13.11.57] and badD; benzoyl-CoA reductase subunit [EC: 1.3.7.8] and these are associated with aminobenzoate and benzoate degradation pathways, respectively (Figure 4.11A). Two were more abundant in soil 2 (catA catechol 1, 2-dioxygenase [EC: 1.13.11.1] and hbaC hcrA 4-hydrobenzoyl-coA reductase subunit) compared to the other soils, from the chlorocyclohexane and chlorobenzene and benzoate degradation pathways (Figure 4.11B). Three were more abundant in soil 3 compared to the other soils (phenol 2-monooxygenase [EC: 1.14.13.7], aliphatic nitrilase [EC:3.5.5.7], and ligA; protocatechuate 4,5-dioxygenase alpha chain) with pathways including chlorocyclohexane and chlorobenzene, and benzoate (Figure 4.11C) and finally three were more

abundant in soil 4 compared to the other soils (hydrogenase large subunit [E1.12.99.6L] & hydrogenase small subunit [E1.12.99.6S, vanA; vanillate monooxygenase [EC 1.14.13.82]], and badE; benzoyl-CoA reductase subunit [EC:1.3.7.8]) with pathways comprising nitrotoluene, aminobenzoate, and benzoate (Figure 4.11D).

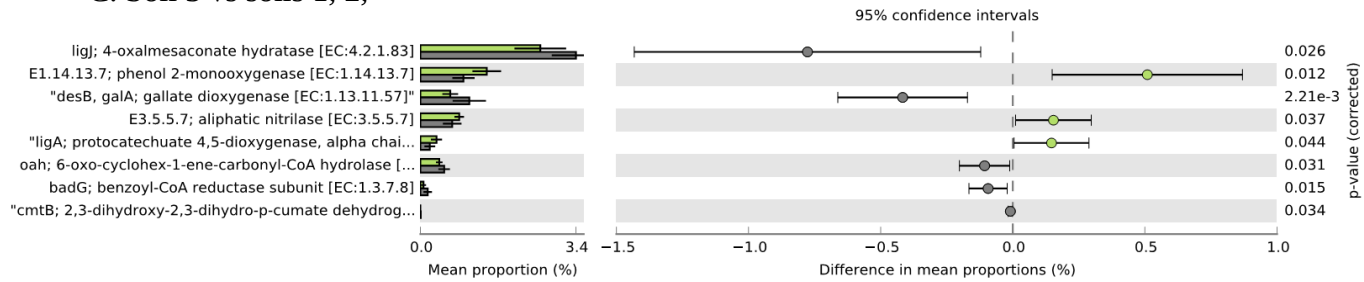
A. Soil 1 vs soils 2, 3, and 4



B. Soil 2 vs soils 1, 3, and



C. Soil 3 vs soils 1, 2,



D. Soil 4 vs soils 1, 2, and

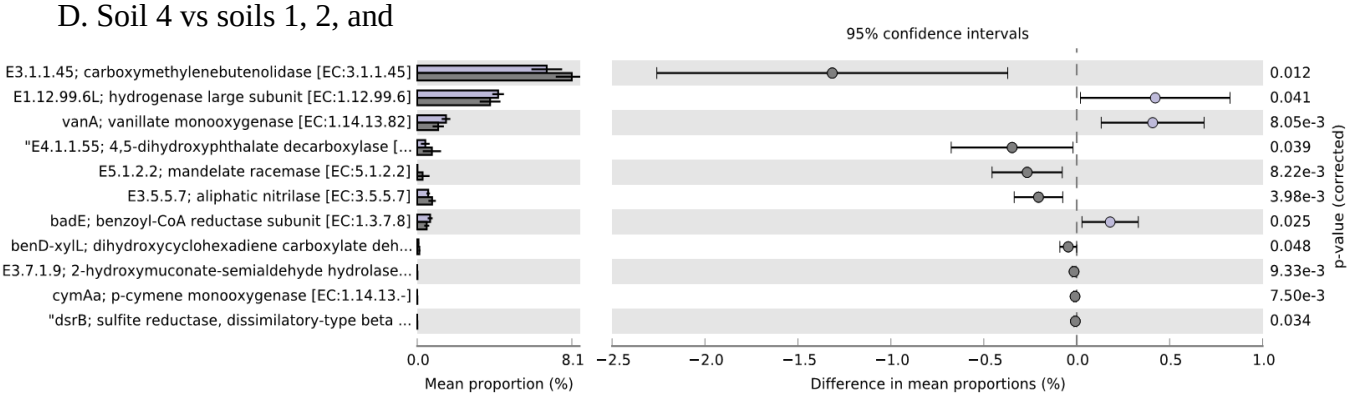


Figure 4.11 Plot comparing each soil vs. the rest of the soils for all of the genes associated with the xenobiotic degrading pathways. Values on the left side of the dotted line indicate the rest of the soils and the values on right indicate the individual soil.

4.5. Discussion

In previous studies (Thelusmond et al., 2018; Thelusmond et al., 2016), 16S rRNA gene amplicon sequencing was coupled with a computational approach (phylogenetic investigation of communities by reconstruction of unobserved states or PICRUSt) to determine the predicted metagenomes in soils following exposure to TCC and CBZ. PICRUSt makes use of evolutional modeling to predict the functional composition of a metagenome using 16S rRNA gene data and a database of reference genomes (Langille et al., 2013). In the current study, the use of shotgun (or whole genome) sequencing was explored to determine both the taxonomic and functional profiles of four soil communities following exposure to one pharmaceutical and two antimicrobial agents. This approach involves the sequencing of all DNA (compared to only the 16S rRNA gene) and therefore should provide a more accurate representation of the potential functional abilities of soil microbial communities.

CBZ, TCC and TCS biodegradation trends found in the current study are similar to those reported by others. Specifically, CBZ and TCC removal in all four soils was slow, consistent with trends others have reported (Dalkmann et al., 2014; Duran-Alvarez et al., 2015; Grossberger et al., 2014; Thelusmond et al., 2018; Thelusmond et al., 2016; Walters et al., 2010) (Snyder et al., 2010; Thelusmond et al., 2018; Wu et al., 2009; Ying et al., 2007). Also, the fast TCS transformation found in the current study is in line with previous reports, which indicated TCS half-lives between 2 and 18 days (Kwon et al., 2010; Ying et al., 2007).

Overall, the phylotypes that were positively impacted during exposure to CBZ or TCC in the current study were different from those previously identified (Thelusmond et al., 2018; Thelusmond et al., 2016). The varying results are likely a result of various factors. Firstly, different analyses methods were used previously (16S rRNA amplicon sequencing and Mothur

(Schloss et al., 2009) analysis of the sequencing data) compared to the current study (shotgun sequencing and MG-RAST analysis of the sequencing data). Also, different soils were used between studies and, in some cases, different concentrations and incubations times were adopted. Considering previous and current datasets, it appears that these chemicals impact a range of genera from numerous phyla (depending on the soil, incubation conditions and analysis approach). Strong trends relating specific phylotypes to CBZ or TCC degradation did not emerge when all datasets were examined together. Relative to our first objective (to determine if specific phylotypes could be associated with CBZ, TCC and TCS degradation in four agricultural soils), several phylotypes were enriched following exposure to each chemical; however, it is unclear if these results will be applicable to other soils.

A second approach was also adopted to determine the microorganisms that could be associated with CBZ, TCC or TCS biodegradation and this involved examining the sequencing data for the abundance of known degrading isolates (at the genus level only). From this analysis, *Rhodococcus* (CBZ), *Streptomyces* (CBZ), *Pseudomonas* (CBZ, TCC, TCS), *Sphingomonas* (CBZ), *Methylobacillus* (TCS) and *Stenotrophomonas* (TCS) were among the most abundant (chemical previously reported to be degraded is shown in parenthesis). These genera were present in both the sample and live control microcosms at similar levels which likely suggests the amended concentrations were not high enough to sustain growth.

A second objective was to compare the occurrence of genes associated with a group of KEGG (Kanehisa, 2002) xenobiotic degradation pathways between the microcosms amended with CBZ, TCC and TCS and the live controls (no chemical added) to determine if specific functional genes could be linked to biodegradation. The results indicated only two or three genes

were impacted for each chemical, again suggesting the concentrations were not high enough to significantly impact the microbial communities.

A third objective was to compare the phylotypes and functional genes associated with a set of xenobiotic degrading pathways between soils to determine if the four different cropping regimes impacted the functional abilities of the soil microbial communities. The majority of gene sequences classified within five xenobiotic degrading KEGG pathways, including those encoding for aminobenozoate, benzoate, chlorocyclohexane and chlorobenzene, dioxin, and nitrotoluene degradation and this is consistent with findings of a recent investigation on the biodegradation of xenobiotics in agricultural soils (Singh et al., 2018). Similar to previous findings, the greatest number of genes was associated with the benzoate degrading pathway (Singh et al., 2018). This finding is not surprising given that benzoate and the associated congeners are the transformation products for a number of xenobiotic compounds (Sikdar and Irvine, 1998). Of the six most abundant genes linked to benzoate degradation found in the current study, four (*badA, pcaC, ligJ*, and *pcaB*) were previously identified as xenobiotic biodegrading genes in wheat rhizosphere (Singh et al., 2018). Similarly, *pcaH* (also highly abundant in the current study from the benzoate pathway) which is responsible for catalyzing ring cleavage in aromatic compounds (Buchan et al., 2000; Eulberg et al., 1998), was abundant in other soil microbial communities screened by real-time PCR (El Azhari et al., 2008). From the other xenobiotic degrading pathways, carboxymethylenebutenolidase (the most abundant gene from the chlorocyclohexane/chlorobenzene pathway) and hydrogenase large subunit (most abundant from the nitrotoluene pathway), were also previously identified in agricultural soils (Singh et al., 2018). The genera *Bradyrhizobium, Rhodopseudomonas, Burkholderia, Candidatus Solibacter, Streptomyces, Mycobacterium* and *Cupriavidus* contained the largest number of genes

associated with the five xenobiotic degrading pathways and are likely important phylotypes for the degradation of organics in these soils.

Although the soils were under four different cropping regimes, there were minimal differences between the phylotypes associated with each of the five xenobiotic degrading pathways. A more detailed comparison of the xenobiotic degrading genes between soils did result in significant differences between the soils for a small number of xenobiotic degrading genes.

4.6. Conclusion

The removal patterns confirm the recalcitrance of CBZ and TCC and the susceptibility of TCS to biodegradation in soils. Clear trends relating specific phylotypes to CBZ or TCC degradation did not emerge when the current dataset was compared to previous datasets, suggesting numerous phyla are impacted by the presence of each chemical, with specific results likely depending on the experimental approach. From the dominant KEGG xenobiotic degrading pathways (aminobenzoate, benzoate, chlorocyclohexane and chlorobenzene, dioxin and nitrotoluene degradation pathways), the benzoate degrading pathway contained the greatest number of genes (forty-two) in these soils. Several genera, most commonly *Bradyrhizobium*, *Mycobacterium, Rhodopseudomonas, Pseudomonas, Cupriavidus,* and *Streptomyces* were associated with these xenobiotic degradation pathways. An important finding concerns the presence of many genera previously associated with CBZ, TCC or TCS degradation (*Rhodococcus*, *Starkeya*, *Pseudomonas*, *Streptomyces*, *Rhizobium*, *Sphingomonas*, *Ochrobactrum*, *Methylobacillus*, *Alicycliphilus* and *Stenotrophomonas*) in each of the four soils. However, additional research is needed to determine if the strains present in these soils are also capable of PPCP biodegradation.

APPENDIX

Appendix

Suplementary Table 4.1 Precursor, product ions and optimized MS/MS parameters for CBZ, TCC and TCS quantification.

Compound	Precursor ion> product ion	CPV(kV)[a]	CV (V)[b]	CE (V)[c]	ST (°C)[d]	DT (°C)[e]	CGF(L/hr)[f]	DGF(L/hr)[g]	CoGF (ml/min)[h]
CBZ	237>194	3.6	22	22	150	350	20	800	2
CBZ-d_{10}	247>204	3.6	22	22	150	350	20	800	2
TCC	313>160	2.5	34	16	130	350	20	800	0.15
TCC-$^{13}C_6$	319>160	2.5	34	17	130	350	20	800	0.15
TCS	287>35	2.5	30	15	130	350	20	800	0.15
TCS-d_3	289>35	2.5	30	15	130	350	20	794	0.15

a: capillary voltage; b: cone voltage; c: collision energy; d: source temperature; e: desolvation temperature; f: cone gas flow; g: desolvation gas flow; h: collision gas flow.

Supplementary Table 4.2 CBZ, TCC and TCS average percent recovery (%) (n=3) in soils 1, 2, 3, and 4.

Soil	CBZ	TCC	TCS
1	83±7	93±3	93±3
2	80±2	90±7	87±3
3	85±3	100±14	93±3
4	96±7	94±8	97±12

Supplementary Table 4.3 MG-RAST sequence data summary

Soil	Chemical	MG-RAST ID	Category	Pre-QC: Sequence Counts	Post QC: Sequence Counts	Post QC: Mean Sequence Length
1	CBZ	mmg4778703.3	Control	5,201,368	4,521,335	230 ± 37 bp
2	CBZ	mmg4778919.3	Control	4,811,760	4,289,132	228 ± 38 bp
3	CBZ	mmg4778708.3	Control	5,060,968	4,472,954	228 ± 38 bp
4	CBZ	mmg4778711.3	Control	5,293,978	4,629,847	230 ± 38 bp
1	CBZ	mmg4780030.3	Sample	5,211,671	4,497,081	232 ± 37 bp
2	CBZ	mmg4778794.3	Sample	5,136,862	4,534,496	231 ± 37 bp
3	CBZ	mmg4778844.3	Sample	4,408,678	3,896,064	232 ± 37 bp
4	CBZ	mmg4779082.3	Sample	6,980,214	6,169,526	229 ± 38 bp
1	TCC	mmg4778704.3	Control	4,352,599	3,783,841	229 ± 38 bp
2	TCC	mmg4778706.3	Control	5,048,021	4,503,356	228 ± 38 bp
3	TCC	mmg4778709.3	Control	5,748,449	5,084,381	228 ± 38 bp
4	TCC	mmg4778712.3	Control	5,219,494	4,580,485	229 ± 38 bp
1	TCC	mgm4778715.3	Sample	6,027,521	5,185,396	230 ± 37 bp
2	TCC	mmg4778935.3	Sample	5,808,107	5,120,017	229 ± 37 bp
3	TCC	mmg4778850.3	Sample	6,014,260	5,309,912	229 ± 38 bp
4	TCC	mmg4778861.3	Sample	5,722,385	5,021,996	230 ± 37 bp
1	TCS	mmg4778705.3	Control	4,344,097	3,774,944	230 ± 38 bp
2	TCS	mmg4778707.3	Control	4,379,711	3,911,788	229 ± 38 bp
3	TCS	mmg4778710.3	Control	4,184,565	3,689,209	229 ± 38 bp
4	TCS	mmg4778713.3	Control	5,516,444	4,814,277	227 ± 38 bp
1	TCS	mmg4778801.3	Sample	5,340,267	4,617,966	228 ± 38 bp
2	TCS	mmg4778851.3	Sample	5,822,938	5,131,236	224 ± 39 bp
3	TCS	mmg4778852.3	Sample	4,552,406	4,016,793	230 ± 37 bp
4	TCS	mmg4778993.3	Sample	6,445,101	5,670,670	230 ± 37 bp

Supplementary Table 4.4 Phylotypes with a statistically significantly different level of abundance in all CBZ amended soils compared to the controls by day 80.

Phylum	**Class**	**Order**	**Family**	**Genus**	**Enriched in**
*	*	*Zoopagales*	*Piptocephalidaceae*	*Piptocephalis*	Controls
Aquificae	*Aquificae (class)*	*Aquificales*	*Hydrogenothermaceae*	*Sulfurihydrogenibium*	**Samples**
Streptophyta	**	*Fabales*	*Fabaceae*	*Glycine*	**Samples**
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	***	Controls
Actinobacteria	*Actinobacteria (class)*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Gardnerella*	Controls
Firmicutes	*Bacilli*	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	Controls
*	*	*Entomophthorales*	*Basidiobolaceae*	*Basidiobolus*	**Samples**
Tenericutes	*Mollicutes*	*Entomoplasmatales*	*Entomoplasmataceae*	*Mesoplasma*	Controls
Proteobacteria	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Pantoea*	Controls
****	****	****	*Hexamitidae*	*Giardia*	Controls
Streptophyta	**	*Caryophyllales*	*Amaranthaceae*	*Spinacia*	**Samples**
****	****	*Kinetoplastida*	*Trypanosomatidae*	*Leishmania*	**Samples**
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Hyphomonadaceae*	*Maricaulis*	**Samples**
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Cytophagaceae*	*Cytophaga*	**Samples**

**unclassified (derived from Fungi)*
***unclassified (derived from Streptophyta)*
****unclassified (derived from Lachnospiraceae)*
*****unclassified (derived from Eukaryota)*

Supplementary Table 4.5 Phylotypes with a statistically significantly different level of abundance in all TCC amended soils compared to the controls by day 80.

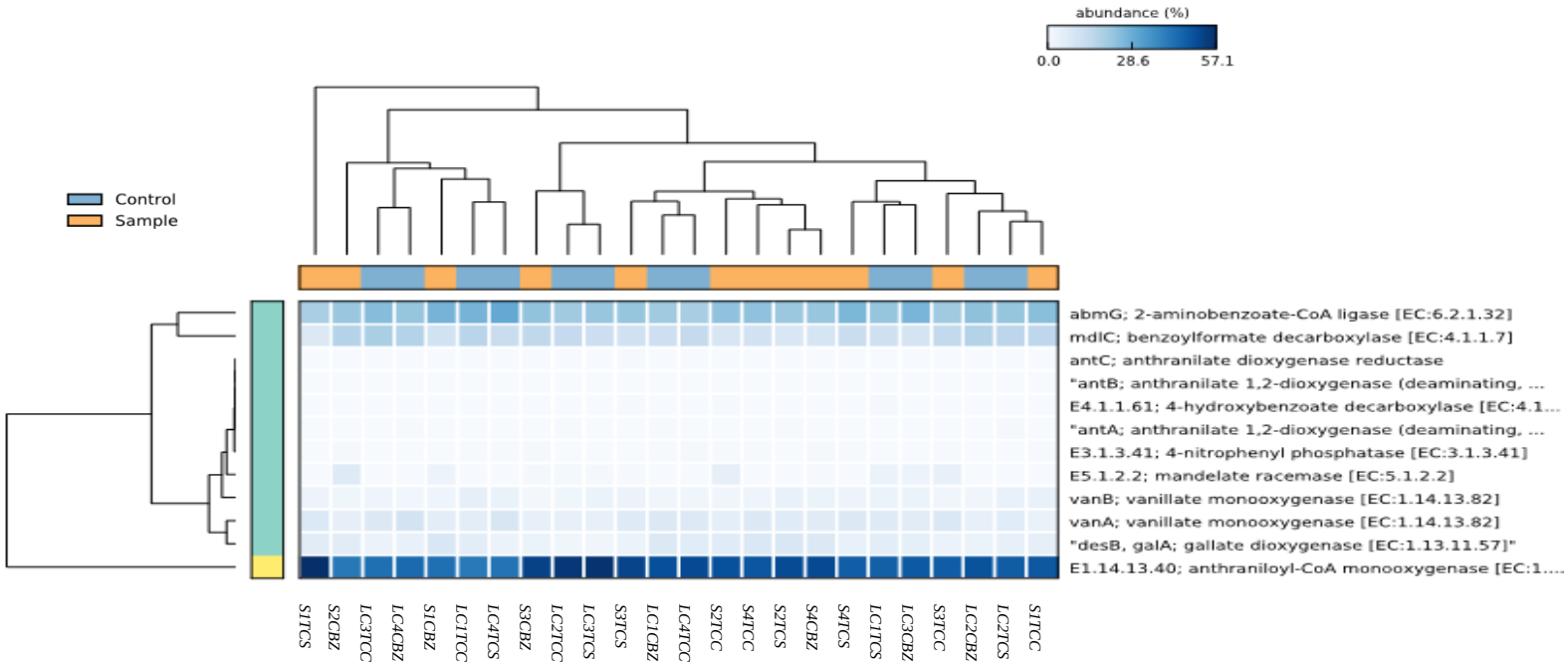
Phylum	**Class**	**Order**	**Family**	**Genus**	**Enriched in**
*	*	*Zoopagales*	*Helicocephalidaceae*	*Rhopalomyces*	Controls
Chordata	*Mammalia*	*Primates*	*Cercopithecidae*	*Macaca*	**Samples**
Arthropoda	*Insecta*	*Phthiraptera*	*Pediculidae*	*Pediculus*	**Samples**
Ascomycota	*Leotiomycetes*	*Helotiales*	*Sclerotiniaceae*	*Botryotinia*	**Samples**
Proteobacteria	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	*Aggregatibacter*	**Samples**
Proteobacteria	*Gammaproteobacteria*	*Thiotrichales*	*Francisellaceae*	*Francisella*	**Samples**
Ascomycota	*Eurotiomycetes*	*Eurotiales*	*Trichocomaceae*	*Neosartorya*	**Samples**
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Alcaligenaceae*	*Bordetella*	Controls
Microsporidia	**	**	*Nosematidae*	*Nosema*	Controls
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Herbaspirillum*	Controls
Proteobacteria	*Alphaproteobacteria*	*Rickettsiales*	*Anaplasmataceae*	*Ehrlichia*	**Samples**
Chordata	*Aves*	*Passeriformes*	*Estrildidae*	*Taeniopygia*	**Samples**
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Oxalobacter*	Controls
Ascomycota	*Eurotiomycetes*	*Eurotiales*	*Trichocomaceae*	*Talaromyces*	**Samples**
Proteobacteria	*Betaproteobacteria*	*Neisseriales*	*Neisseriaceae*	*Kingella*	Controls
Proteobacteria	*Betaproteobacteria*	*Neisseriales*	*Neisseriaceae*	*Lutiella*	Controls
***	*Cryptophyta*	*Cryptomonadales*	*Cryptomonadaceae*	*Cryptomonas*	Controls
Thermotogae	*Thermotogae (class)*	*Thermotogales*	*Thermotogaceae*	*Petrotoga*	Controls
Nanoarchaeota	****	****	****	*Nanoarchaeum*	**Samples**

**unclassified (derived from Fungi)*
***unclassified (derived from Microsporidia)*
****unclassified (derived from Eukaryota)*
*****unclassified (derived from Nanoarchaeota)*

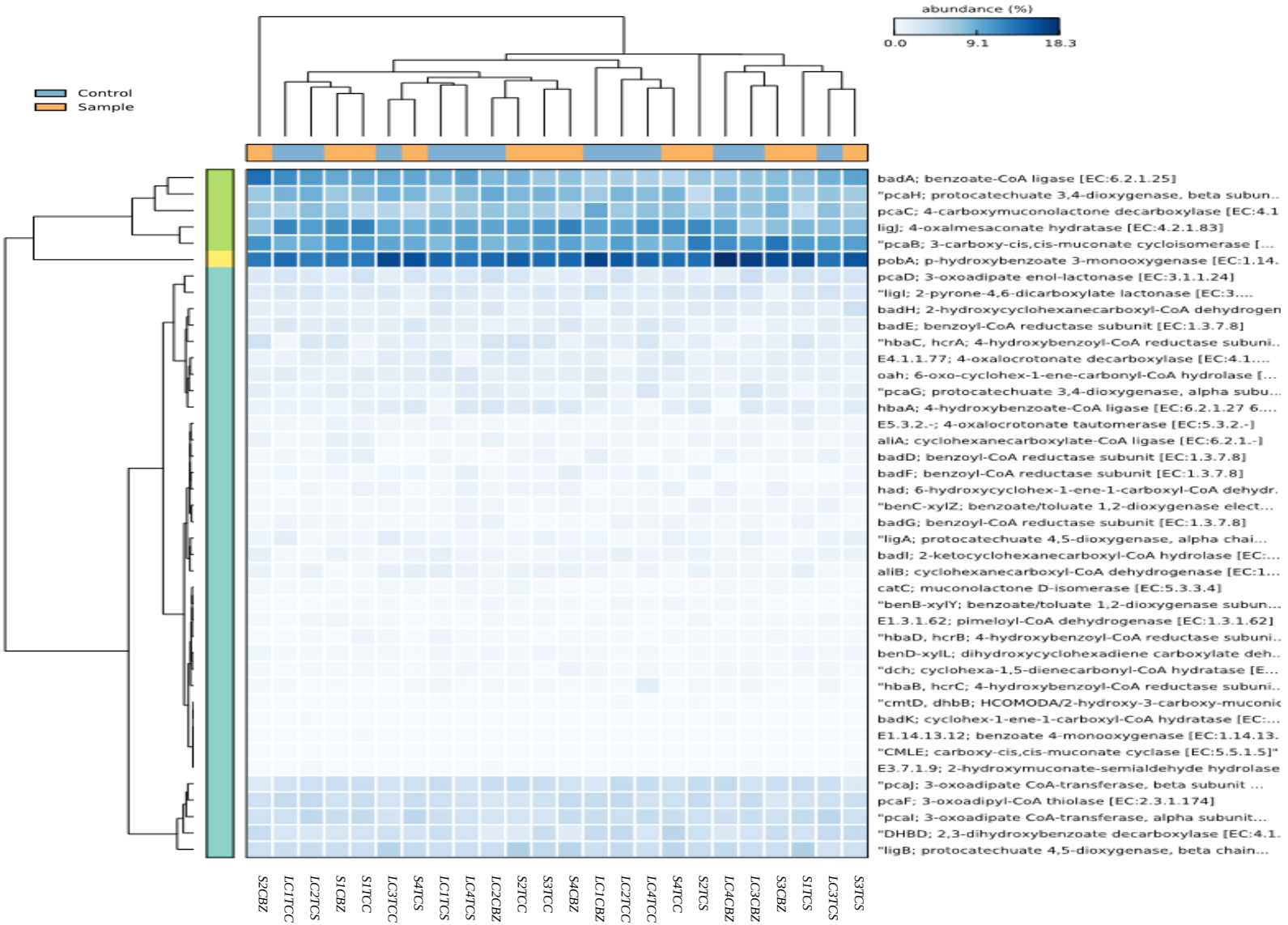
Supplementary Table 4.6 Phylotypes with a statistically significantly different level of abundance in all TCS amended soils compared to the controls by day 15.

Phylum	**Class**	**Order**	**Family**	**Genus**	**Enriched in**
Ascomycota	*Saccharomycetes*	*Saccharomycetales*	*Saccharomycetaceae*	*Kluyveromyces*	Controls
Proteobacteria	*Gammaproteobacteria*	*Thiotrichales*	*Thiotrichaceae*	*Beggiatoa*	**Samples**
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	**Samples**
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Burkholderiaceae*	*Polynucleobacter*	**Samples**
*	*	*Zoopagales*	*Helicocephalidaceae*	*Rhopalomyces*	Controls
Proteobacteria	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	*Mannheimia*	**Samples**
Cnidaria	*Anthozoa*	*Scleractinia*	*Pocilloporidae*	*Seriatopora*	Controls
Arthropoda	*Arachnida*	*Ixodida*	*Ixodidae*	*Ixodes*	**Samples**
Ascomycota	*Saccharomycetes*	*Saccharomycetales*	*Saccharomycetaceae*	*Zygosaccharomyces*	**Samples**
Chlamydiae	*Chlamydiae (class)*	*Chlamydiales*	*Waddliaceae*	*Waddlia*	**Samples**
Cyanobacteria	**	*Oscillatoriales*	***	*Arthrospira*	**Samples**
Ascomycota	*Eurotiomycetes*	*Eurotiales*	*Trichocomaceae*	*Aspergillus*	**Samples**
Bacillariophyta	*Fragilariophyceae*	*Fragilariales*	*Fragilariaceae*	*Synedra*	**Samples**

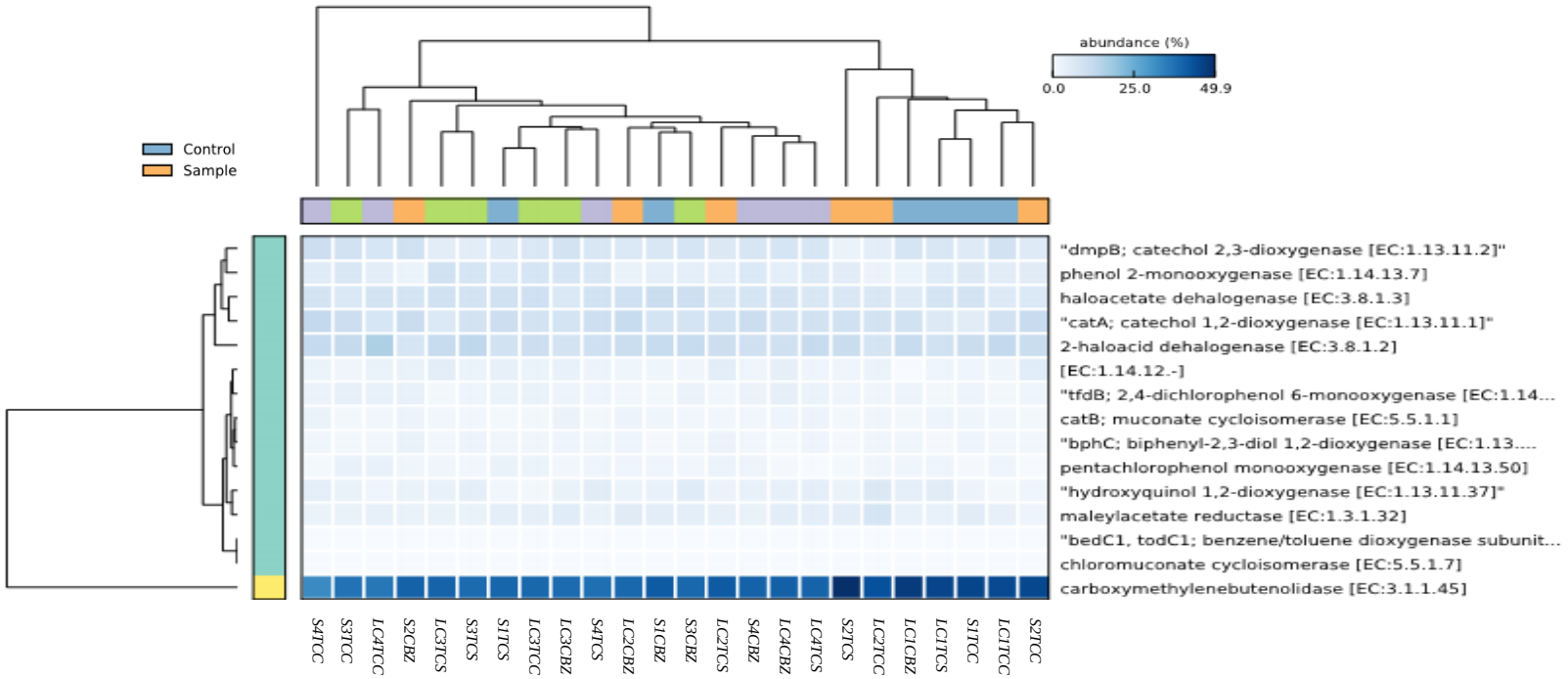
**unclassified (derived from Fungi)*
***unclassified (derived from Cyanobacteria)*
****unclassified (derived from Oscillatoriales*



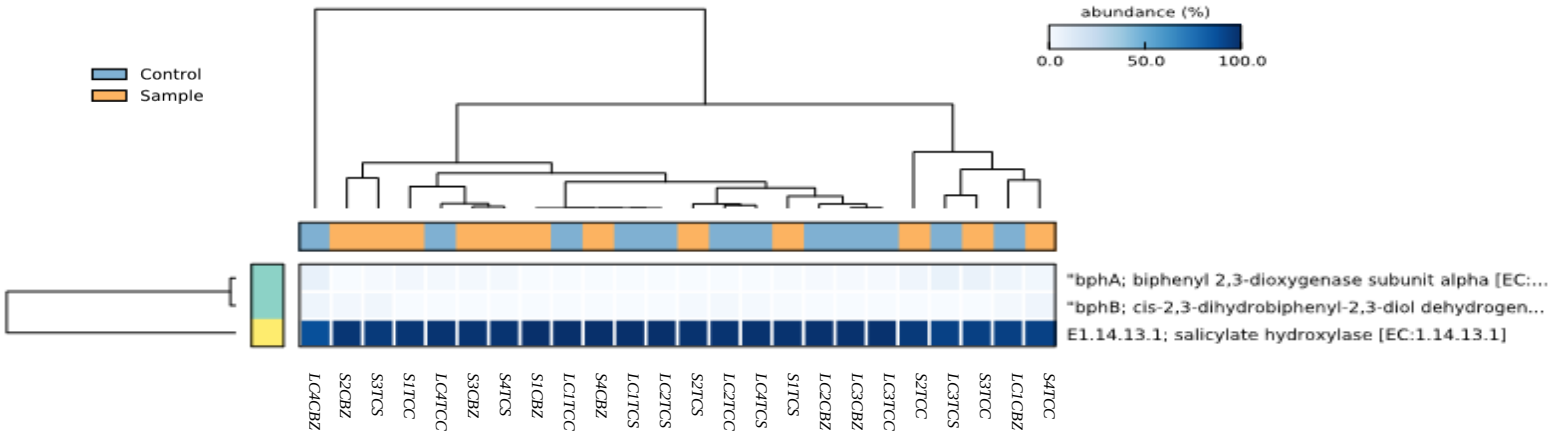
Supplementary Figure 4.1 Heatmaps of the genes associated with pathways encoding for aminobenzoate biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for all four soils combined at day 80 (CBZ or TCC) and day 15 (TCS).



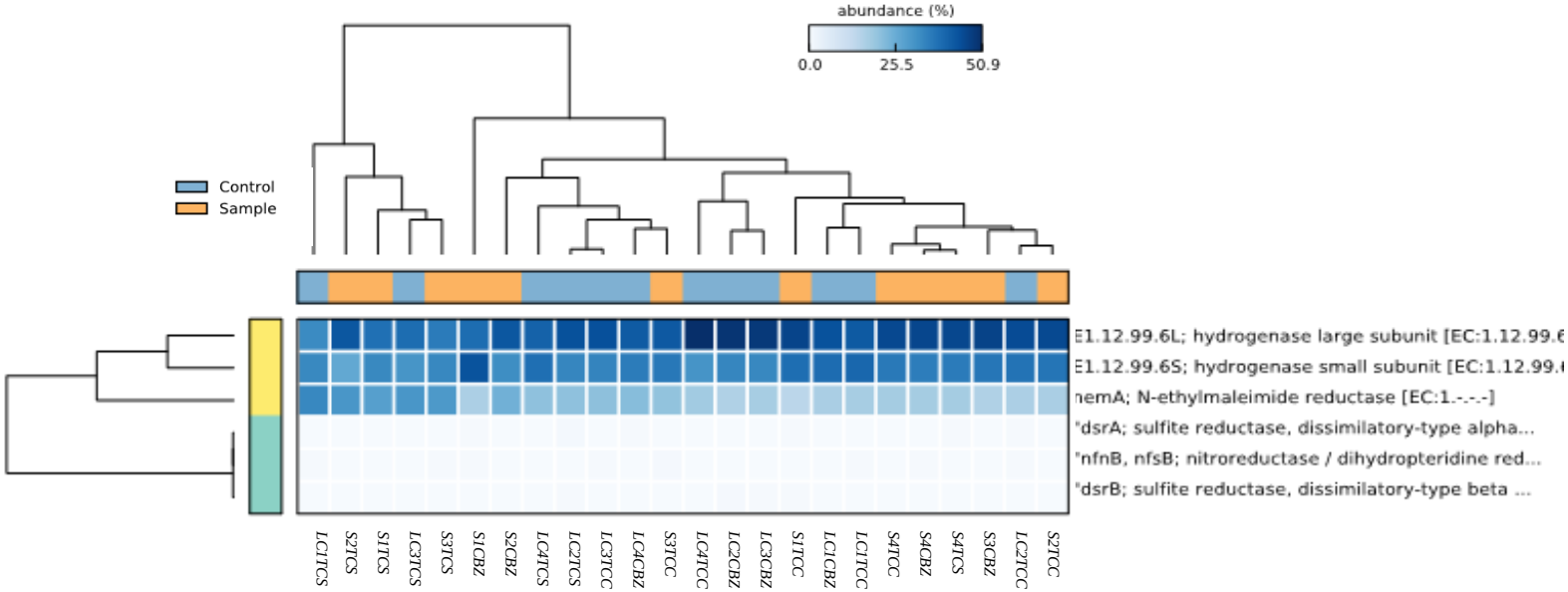
Supplementary Figure 4.2 Heatmaps of the genes associated with pathways encoding for benzoate biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for all four soils combined at day 80 (CBZ or TCC) and day 15 (TCS).



Supplementary Figure 4.3 Heatmaps of the genes associated with pathways encoding for chlorocyclohexane and chlorobenzene biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for all four soils combined at day 80 (CBZ or TCC) and day 15 (TCS).



Supplementary Figure 4.4 Heatmaps of the genes associated with pathways encoding for dioxin biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for all four soils combined at day 80 (CBZ or TCC) and day 15 (TCS).



Supplementary Figure 4.5 Heatmaps of the genes associated with pathways encoding for nitrotoluene biodegradation in the samples amended with CBZ, TCC, or TCS and the live controls (no CBZ, TCC or TCS) for all four soils combined at day 80 (CBZ or TCC) and day 15 (TCS).

REFERENCES

REFERENCES

Al-Rajab, A.J., Sabourin, L., Lapen, D.R., Topp, E. 2015. Dissipation of triclosan, triclocarban, carbamazepine and naproxen in agricultural soil following surface or sub-surface application of dewatered municipal biosolids. Science of the Total Environment. 512, 480-488.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International. 86, 412-431.

Barber, L.B., Murphy, S.F., Verplanck, P.L., Sandstrom, M.W., Taylor, H.E., Furlong, E.T. 2006. Chemical loading into surface water along a hydrological, biogeochemical, and land use gradient: A holistic watershed approach. Environmental Science & Technology. 40, 475-486.

Bendz, D., Paxeus, N.A., Ginn, T.R., Loge, F.J. 2005. Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Hoje River in Sweden. Journal of Hazardous Materials. 122, 195-204.

Bessa, V.S., Moreira, I.S., Tiritan, M.E., Castro, P.M.L. 2017. Enrichment of bacterial strains for the biodegradation of diclofenac and carbamazepine from activated sludge. International Biodeterioration & Biodegradation. 120, 135-142.

Bester, K. 2005. Fate of triclosan and triclosan-methyl in sewage treatment plants and surface waters. Archives of Environmental Contamination and Toxicology. 49, 9-17.

Boyd, G.R., Palmeri, J.M., Zhang, S.Y., Grimm, D.A. 2004. Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana, USA. Science of the Total Environment. 333, 137-148.

Braganca, I., Placido, A., Paiga, P., Domingues, V.F., Delerue-Matos, C. 2012. QuEChERS: a new sample preparation approach for the determination of ibuprofen and its metabolites in soils. Science of the Total Environment. 433, 281-289.

Buchan, A., Collier, L.S., Neidle, E.L., Moran, M.A. 2000. Key aromatic-ring-cleaving enzyme, protocatechuate 3,4-dioxygenase, in the ecologically important marine Roseobacter lineage. Applied and Environmental Microbiology. 66, 4662-4672.

Cha, J.M., Cupples, A.M. 2009. Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids. Water Research. 43, 2522-2530.

Cha, J.M., Cupples, A.M. 2010. Triclocarban and triclosan biodegradation at field concentrations and the resulting leaching potentials in three agricultural soils. Chemosphere. 81, 494-499.

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

Chu, S., Metcalfe, C.D. 2007. Simultaneous determination of triclocarban and triclosan in municipal biosolids by liquid chromatography tandem mass spectrometry. Journal of Chromatography A. 1164, 212-218.

Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., Kroiss, H. 2005. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. Water Research. 39, 4797-4807.

Cox, M.P., Peterson, D.A., Biggs, P.J. 2010. SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data. Bmc Bioinformatics. 11:485, 1-6.

Dalkmann, P., Siebe, C., Amelung, W., Schloter, M., Siemens, J. 2014a. Does long-term irrigation with untreated wastewater accelerate the dissipation of pharmaceuticals in soil? Environmental Science & Technology. 48, 4963-4970.

Dalkmann, P., Siebe, C., Amelung, W., Schloter, M., Siemens, J. 2014b. Does long-term irrigation with untreated wastewater accelerate the dissipation of pharmaceuticals in soil? Environmental Science & Technology. 48, 4963-4970.

Ding, Y.J., Zhang, W.H., Gu, C., Xagoraraki, I., Li, H. 2011. Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. Journal of Chromatography A. 1218, 10-16.

Duran-Alvarez, J.C., Prado, B., Gonzalez, D., Sanchez, Y., Jimenez-Cisneros, B. 2015. Environmental fate of naproxen, carbamazepine and triclosan in wastewater, surface water and wastewater irrigated soil-results of laboratory scale experiments. Science of the Total Environment. 538, 350-362.

El Azhari, N., Bru, D., Sarr, A., Martin-Laurent, F. 2008. Estimation of the density of the protocatechuate-degrading bacterial community in soil by real-time PCR. European Journal of Soil Science. 59, 665-673.

Eulberg, D., Lakner, S., Golovleva, L.A., Schlomann, M. 1998. Characterization of a protocatechuate catabolic gene cluster from *Rhodococcus opacus* 1CP: Evidence for a merged enzyme with 4-carboxymuconolactone-decarboxylating and 3-oxoadipate enol-lactone-hydrolyzing activity. Journal of Bacteriology. 180, 1072-1081.

Gauthier, H., Yargeau, V., Cooper, D.G. 2010. Biodegradation of pharmaceuticals by *Rhodococcus rhodochrous* and *Aspergillus niger* by co-metabolism. Science of the Total Environment. 408, 1701-1706.

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

Gottschall, N., Topp, E., Metcalfe, C., Edwards, M., Payne, M., Kleywegt, S., et al. 2012. Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field. Chemosphere. 87, 194-203.

Grossberger, A., Hadar, Y., Borch, T., Chefetz, B. 2014a. Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. Environmental Pollution. 185, 168-177.

Grossberger, A., Hadar, Y., Borch, T., Chefetz, B. 2014b. Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. Environmental Pollution. 185, 168-177.

Haggard, B.E., Galloway, J.M., Green, W.R., Meyer, M.T. 2006. Pharmaceuticals and other organic chemicals in selected north-central and northwestern Arkansas streams. Journal of Environmental Quality. 35, 1078-1087.

Halden, R.U., Paull, D.H. 2005. Co-occurrence of triclocarban and triclosan in US water resources. Environmental Science & Technology. 39, 1420-1426.

Heidler, J., Halden, R.U. 2007. Mass balance assessment of triclosan removal during conventional sewage treatment. Chemosphere. 66, 362-369.

Hua, W.Y., Bennett, E.R., Letcher, R.J. 2005. Triclosan in waste and surface waters from the upper Detroit River by liquid chromatography-electrospray-tandem quadrupole mass spectrometry. Environment International. 31, 621-630.

Kanda, R., Griffin, P., James, H.A., Fothergill, J. 2003. Pharmaceutical and personal care products in sewage treatment works. Journal of Environmental Monitoring. 5, 823-830.

Kandel, S., Vogel, J., Penn, C., Brown, G. 2017. Phosphorus Retention by Fly Ash Amended Filter Media in Aged Bioretention Cells. Water. 9.

Kanehisa, M. 2002. The KEGG database. In Silico Simulation of Biological Processes. 247, 91-103.

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

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., et al. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental Science & Technology. 36, 1202-1211.

Kwon, L.W., Armbrust, K.L., Xia, K. 2010. Transformation of triclosan and triclocarban in soils and biosolids-applied soils. Journal of Environmental Quality. 39, 1139-1144.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., et al. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology. 31, 814-821.

Lee, D.G., Zhao, F., Rezenom, Y.H., Russell, D.H., Chu, K.H. 2012. Biodegradation of triclosan by a wastewater microorganism. Water Research. 46, 4226-34.

Li, A., Cai, R., Cui, D., Qiu, T., Pang, C.L., Yang, J.X., et al. 2013. Characterization and biodegradation kinetics of a new cold-adapted carbamazepine-degrading bacterium, *Pseudomonas* sp CBZ-4. Journal of Environmental Sciences (China). 25, 2281-2290.

Lolas, I.B., Chen, X.J., Bester, K., Nielsen, J.L. 2012. Identification of triclosan-degrading bacteria using stable isotope probing, fluorescence in situ hybridization and microautoradiography. Microbiology-Sgm. 158, 2796-2804.

Loraine, G.A., Pettigrove, M.E. 2006. Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California. Environmental Science & Technology. 40, 687-695.

McAvoy, D.C., Schatowitz, B., Jacob, M., Hauk, A., Eckhoff, W.S. 2002. Measurement of triclosan in wastewater treatment systems. Environmental Toxicology and Chemistry. 21, 1323-1329.

Meade, M.J., Waddell, R.L., Callahan, T.M. 2001. Soil bacteria Pseudomonas putida and Alcaligenes xylosoxidans subsp. denitrificans inactivate triclosan in liquid and solid substrates. FEMS Microbiology Letters. 204, 45-8.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., Kubal, M., et al. 2008. The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. Bmc Bioinformatics. 9.

Miao, X.S., Metcalfe, C.D. 2003. Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography-electrospray tandem mass spectrometry. Analytical Chemistry. 75, 3731-3738.

Miller, T.R., Heidler, J., Chillrud, S.N., Delaquil, A., Ritchie, J.C., Mihalic, J.N., et al. 2008. Fate of triclosan and evidence for reductive dechlorination of triclocarban in estuarine sediments. Environmental Science & Technology. 42, 4570-4576.

Monteiro, S.C., Boxall, A.B.A. 2009. Factors affecting the degradation of pharmaceuticals in agricultural soils. Environmental Toxicology and Chemistry. 28, 2546-2554.

Morais, S.A., Delerue-Matos, C., Gabarrell, X., Blanquez, P. 2013. Multimedia fate modeling and comparative impact on freshwater ecosystems of pharmaceuticals from biosolids-amended soils. Chemosphere. 93, 252-262.

Morrall, D., McAvoy, D., Schatowitz, B., Inauen, J., Jacob, M., Hauk, A., et al. 2004. A field study of triclosan loss rates in river water (Cibolo Creek, TX). Chemosphere. 54, 653-660.

Mulla, S.I., Hu, A.Y., Wang, Y.W., Sun, Q., Huang, S.L., Wang, H., et al. 2016a. Degradation of triclocarban by a triclosan-degrading *Sphingomonas* sp strain YL-JM2C. Chemosphere. 144, 292-296.

Mulla, S.I., Wang, H., Sun, Q., Hu, A.Y., Yu, C.P. 2016b. Characterization of triclosan metabolism in Sphingomonas sp strain YL-JM2C. Scientific Reports. 6.

Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G. 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 30, 3123-3124.

Paz, A., Tadmor, G., Malchi, T., Blotevogel, J., Borch, T., Polubesova, T., et al. 2016. Fate of carbamazepine, its metabolites, and lamotrigine in soils irrigated with reclaimed wastewater: Sorption, leaching and plant uptake. Chemosphere. 160, 22-29.

Popa, C., Favier, L., Dinica, R., Semrany, S., Djelal, H., Amrane, A., et al. 2014. Potential of newly isolated wild *Streptomyces* strains as agents for the biodegradation of a recalcitrant pharmaceutical, carbamazepine. Environmental Technology. 35, 3082-3091.

Pruitt, K.D., Tatusova, T., Maglott, D.R. 2005. NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Research. 33, D501-D504.

Radjenovic, J., Jelic, A., Petrovic, M., Barcelo, D. 2009. Determination of pharmaceuticals in sewage sludge by pressurized liquid extraction (PLE) coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Analytical and Bioanalytical Chemistry. 393, 1685-1695.

Rho, M.N., Tang, H.X., Ye, Y.Z. 2010. FragGeneScan: predicting genes in short and error-prone reads. Nucleic Acids Research. 38.

Robertson, P.G., Hamilton, S.K. 2015. Long-term ecological research in agricultural landscapes at the Kellogg Biological Station LTER site: conceptual and experimental framework. Oxford University Press, New York, 1-32.

Sabaliunas, D., Webb, S.F., Hauk, A., Jacob, M., Eckhoff, W.S. 2003. Environmental fate of Triclosan in the River Aire Basin, UK. Water Research. 37, 3145-3154.

Salvia, M.V., Vulliet, E., Wiest, L., Baudot, R., Cren-Olive, C. 2012. Development of a multi-residue method using acetonitrile-based extraction followed by liquid chromatography-tandem mass spectrometry for the analysis of steroids and veterinary and human drugs at trace levels in soil. Journal of Chromatography A. 1245, 122-133.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for

describing and comparing microbial communities. Applied and Environmental Microbiology. 75, 7537-7541.

Sikdar, S.K., Irvine, R.L. 1998. Biodegradation technology developments: principles and practice. Technomic Publishing Company, Inc. 2.

Singer, H., Muller, S., Tixier, C., Pillonel, L. 2002. Triclosan: Occurrence and fate of a widely used biocide in the aquatic environment: Field measurements in wastewater treatment plants, surface waters, and lake sediments. Environmental Science & Technology. 36, 4998-5004.

Singh, D.P., Prabha, R., Gupta, V.K., Verma, M.K. 2018. Metatranscriptome analysis deciphers multifunctional genes and enzymes linked with the degradation of aromatic compounds and pesticides in the wheat rhizosphere. Front Microbiol. 9, 1-15.

Sipahutar, M.K., Piapukiew, J., Vangnai, A.S. 2018. Efficiency of the formulated plant-growth promoting *Pseudomonas fluorescens* MC46 inoculant on triclocarban treatment in soil and its effect on *Vigna radiata* growth and soil enzyme activities. Journal of Hazardous Materials. 344, 883-892.

Sipahutar, M.K., Vangnai, A.S. 2017. Role of plant growth-promoting *Ochrobactrum* sp MC22 on triclocarban degradation and toxicity mitigation to legume plants. Journal of Hazardous Materials. 329, 38-48.

Snyder, E.H., O'Connor, G.A., McAvoy, D.C. 2010. Fate of 14C-triclocarban in biosolids-amended soils. Science of the Total Environment. 408, 2726-2732.

Spongberg, A.L., Witter, J.D. 2008. Pharmaceutical compounds in the wastewater process stream in Northwest Ohio. Science of the Total Environment. 397, 148-157.

Stackelberg, P.E., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Henderson, A.K., Reissman, D.B. 2004. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-watertreatment plant. Science of the Total Environment. 329, 99-113.

Thelusmond, J.R., Kawka, E., Strathmann, T.J., Cupples, A.M. 2018. Diclofenac, carbamazepine and triclocarban biodegradation in agricultural soils and the microorganisms and metabolic pathways affected. Science of the Total Environment. 640, 1393-1410.

Thelusmond, J.R., Strathmann, T.J., Cupples, A.M. 2016. The identification of carbamazepine biodegrading phylotypes and phylotypes sensitive to carbamazepine exposure in two soil microbial communities. Science of the Total Environment. 571, 1241-1252.

Thomas, P.M., Foster, G.D. 2005. Tracking acidic pharmaceuticals, caffeine, and triclosan through the wastewater treatment process. Environmental Toxicology and Chemistry. 24, 25-30.

Thompson, A., Griffin, P., Stuetz, R., Cartmell, E. 2005. The fate and removal of triclosan during wastewater treatment. Water Environment Research. 77, 63-67.

Vazquez-Roig, P., Segarra, R., Blasco, C., Andreu, V., Pico, Y. 2010. Determination of pharmaceuticals in soils and sediments by pressurized liquid extraction and liquid chromatography tandem mass spectrometry. Journal of Chromatography A. 1217, 2471-2483.

Walker, C.W., Watson, J.E., Williams, C. 2012. Occurrence of carbamazepine in soils under different land uses receiving wastewater. Journal of Environmental Quality. 41, 1263-1267.

Walters, E., McClellan, K., Halden, R.U. 2010. Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. Water Research. 44, 6011-6020.

Waltman, E.L., Venables, B.J., Waller, W.Z. 2006. Triclosan in a North Texas wastewater treatment plant and the influent and effluent of an experimental constructed wetland. Environmental Toxicology and Chemistry. 25, 367-372.

Wu, C., Spongberg, A.L., Witter, J.D. 2009. Adsorption and degradation of triclosan and triclocarban in soils and biosolids-amended soils. Journal of Agricultural and Food Chemistry. 57, 4900-4905.

Wu, C., Spongberg, A.L., Witter, J.D., Fang, M., Ames, A., Czajkowski, K.P. 2010. Detection of pharmaceuticals and personal care products in agricultural soils receiving biosolids application. CLEAN - Soil, Air, Water. 38, 230-237.

Wu, X.Q., Dodgen, L.K., Conkle, J.L., Gan, J. 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Science of the Total Environment. 536, 655-666.

Ying, G.G., Kookana, R.S. 2007. Triclosan in wastewaters and biosolids from Australian wastewater treatment plants. Environment International. 33, 199-205.

Ying, G.G., Yu, X.Y., Kookana, R.S. 2007. Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. Environmental Pollution. 150, 300-305.

Yun, H., Liang, B., Kong, D.Y., Li, Z.L., Qi, G.S., Wang, A.J. 2017a. Enhanced biotransformation of triclocarban by *Ochrobactrum* sp TCC-1 under anoxic nitrate respiration conditions. Current Microbiology. 74, 491-498.

Yun, H., Liang, B., Qiu, J., Zhang, L., Zhao, Y., Jiang, J., et al. 2017b. Functional characterization of a novel amidase involved in biotransformation of triclocarban and its dehalogenated congeners in *Ochrobactrum* sp. TCC-2. Environmental Science & Technology. 51, 291-300.

Chapter 5:

Conclusions

In all studies, CBZ was persistent in soils under both aerobic and anaerobic conditions. Under aerobic conditions, CBZ degraded slowly (half-life >~130 days), whereas under anaerobic conditions, CBZ exhibited no decrease in concentration. Unlike CBZ, DCF dissipated rapidly (< 7days). However, DCF was recalcitrant under NO_3^- reducing, SO_4^{2-} reducing, and methanogenic conditions. Similar to CBZ, TCC degradation occurred slowly under aerobic conditions with half-life values of >165 days. In contrast, TCS biodegradation was rapid under aerobic conditions. Comparisons in concentrations between the abiotic controls and live samples indicated the removal was biological. The persistence of CBZ and DCF under anaerobic conditions indicates these two pharmaceuticals will be recalcitrant under water saturated and oxygen depleted environments.

In Chapter 2, for soil 1, several phylotypes were enriched following CBZ degradation compared to the controls, including unclassified *Sphingomonadaceae*, *Xanthomonadaceae* and *Rhodobacteraceae*, as well as *Sphingomonas*, *Aquicella* and *Microvirga*. These phylotypes are considered putative CBZ degraders as they appear to be benefiting from CBZ biodegradation. PICRUSt revealed that soil 1 contained a greater abundance of xenobiotic degrading genes compared to soil 2, and thus, this analysis method offers a potential valuable approach for predicting CBZ attenuation in soils. PICRUSt analysis also implicated *Sphingomonadaceae* and *Xanthomonadaceae* in drug metabolism. Interestingly, numerous phylotypes decreased in abundance following CBZ exposure and these varied with soil type, concentration, duration of exposure, and the availability of oxygen. For three phylotypes (*Flavobacterium, 3 genus incertae*

sedis and unclassified *Bacteroidetes*), the relative abundance was reduced in both soils, indicating a notable sensitivity to CBZ for these microorganisms.

In Chapter 3, DCF aerobic biodegradation contributed to the enrichment of phylotypes including *Gemmatimonas* and *Streptomyces* as well as others in the phyla *Proteobacteria* and *Actinobacteria*. Similarly, phylotypes in the phyla *Bacteroidetes, Actinobacteria Proteobacteria* and *Verrucomicrobia* were enriched upon exposure to CBZ. Likewise, phylotypes in the *Actinobacteria* and *Proteobacteria* increased in abundance as a result of exposure to TCC. While CBZ exhibited minor impacts on the KEGG metabolic pathways, DCF and TCC aerobic biodegradation was associated several KEGG metabolic pathways including those encoding for genes associated with both simple and complex substrates degradation.

Unlike the above mentioned studies where 16S RNA amplicon sequencing was employed to examine the soil microbial community, Chapter 4 involved the use of shotgun to analyze the soil microbial communities. Six phylotypes including *Burkholderia*, *Streptomyces Rhodopseudomonas*, *Mycobacterium*, *Chthonibacter* and *Sorangium* were the most abundant phylotypes in the soils. The degrading KEGG pathways associated with xenobiotic degradation of compounds such as aminobenzoate, benzoate, chlorocyclohexane and chlorobenzene, dioxin and nitrotoluene, were also found for each soil. Among these pathways, benzoate was found to be the most common pathway. To the above pathways were associated a number of xenobiotics degrading genes with those encoded for benzoate degradation being the most abundant followed by the genes encoded for chlorocyclohexane and chlorobenzene degradation. A number of bacterial isolates previously associated with CBZ, TCC, and TCS degradation were also identified in that study. These phylotypes included the following genera: *Rhodococcus*, *Pseudomonas, Streptomyces, Rhizobium, Bordetella*, *Sphingomonas*, *Ochrobactrum*,

Methylobacillus, *Alicycliphilus*, and *Stenotrophomonas*. A number of xenobiotic degrading genes were also identified for each pathway, and the genes encoded for benzoate degradation was the most abundant followed by those encoded for chlorocyclohexane and chlorobenzene degradation.

In summary, this research indicates a number of phylotypes are likely involved in PPCP biodegradation in agricultural soils. Also, the work suggests that the phylotypes impacted are affected by the experimental conditions (e.g. concentration, soil type, incubation time). From the PPCPs examined, CBZ and TCC are highly recalcitrant and will likely remain in agricultural soils for extended periods of time.

5.1. Future Work

Although the biodegradation of the parent PPCPs was observed in the current work, time did not permit the identification of the metabolites formed. Such information is important for elucidating the degradation pathways of these xenobiotics. Furthermore, this information is valuable for assessing the risk associated with these PPCPs, as some metabolites (e.g. CBZ) can be found at higher concentration in the environment than the parent compound. Also, some metabolites may even be more toxic than the parent compound.

An additional future area of research concerns the isolation of putative CBZ, DCF, TCC, and TCS degraders identified in the current study to confirm that are capable of PPCP biodegradation. The information presented here regarding the soil phylotypes linked to CBZ, DCF, TCC, and TCS biodegradation should be used to isolate microorganisms capable of degrading these xenobiotics. In particular, the genus *Pseudomonas* is of special interest since it has been linked to the degradation of CBZ, TCC, and TCS.