Possible Effect of Pesticides and Fertilizers on Soil Microbiomes of a School Campus

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Abstract

Soil bacteria are essential to agriculture, food webs, and perhaps even climate regulation (1,2). Pesticides are an integral part of modern agronomy which has affected microorganisms as well as humans, and lead to poor nutritional value in vegetation. The aim of this research is to analyze the variation of microbiome based on different school campus locations with different probable pesticide/fertilizer applications. It was hypothesized that the population of the bacteria would be different depending on the location. Soil samples were used to prepare DNA for next-generation sequencing to detect microbiome variations. The results show differences in microbiome profiles between the three soil samples, supporting the hypothesis. The AD3 bacteria was significantly higher in the farm, and the least in the golf course. The possible cause could be past usage of pesticides in the regions. It is a concerning outcome because the existence of this bacteria could be beneficial to human health. Also, the differences in microbiome profiles may be used as indicators of the presence of these chemicals in the soil.

Introduction

It is well known that pesticides meant to control insect populations can also affect microorganisms in the soil (1,2). Many years may pass before these chemicals are broken down, and the altered soil microbiome directly impacts human health by decreasing the nutrition value of the crops grown in it (3). Recent studies show that interactions between soil microbiota and plant growth cycles are not well understood, and that a better appreciation of how these systems interact could lead to new breakthroughs in agriculture (4). In 2011, the New York State Child Safe Playing Fields Act was passed after studies showed the many negative impacts of pesticides on children during development. Pesticide application was subsequently banned in New York for all playgrounds, turf, playing fields, and playground equipment (5). The aim of our project was to discover how bacterial populations vary as a function of past and present pesticide use near our school campus. We investigated this by comparing the soil microbiomes of samples from the Longwood campus (where pesticides have not been in use since 2011) to the microbiomes of samples collected from soil bordering a farm and a golf course, where pesticides and fertilizers have most likely been used for many years. We have been able to gain the following information concerning the farm property: from 1989 to 2005, the pesticide Lannate (a broad spectrum insecticide) and the common commercial fungicide Champ were used on that property bordering the school. In addition, we obtained information regarding the use of specific pesticides, fungicides, and herbicides on fifty Long Island golf courses during the 1990's, and we can safely assume that these were used at the golf course close to the school property (6). A new appreciation for the soil microbiome in golf course turf management is beginning to change long-held practices on many golf courses (7).

Materials and Methods

Soil samples were collected using a coring protocol and GPS coordinates were recorded. Samples were frozen at -20oC until DNA extraction. The MoBio DNA Isolation Kit was used for processing the soil sample DNA at an Open Lab session at BNL. For PCR, 2 ul of the DNA was combined with prepared primer mix and Taq polymerase. The PCR temperatures and number of cycles optimum for producing the amplified DNA were entered into a program that ran the cycler, as instructed by our Open Lab leaders. The primer used was designed to amplify a 16S ribosomal DNA region in the samples. A small portion of the amplified DNA was checked for quality by separating it via agarose gel electrophoresis. Samples of high enough quality were sent to a laboratory for Illumina next-generation sequencing.

Results

Nine soil samples were collected from the Longwood high school campus. Triplicates were prepared representing soil from the middle of the school campus, the border of the campus with a farm, and the border of the campus with a golf course. The triplicate samples showed good reproducibility within themselves. Profiles of all samples were similar, but the levels of certain types of bacteria differed in each sample type. All samples contained proteobacteria, actinobacteria, and acidobacteria (Fig 1). In the farm sample, phylum AD3 bacteria were nearly absent, while the school campus and golf course contained this phylum (Fig 2). Golf course samples contained the highest levels of AD3 bacteria, and the lowest levels of planctomycetes bacteria (Fig 2). School campus samples contained the highest levels of chloroflexi bacteria (Fig 2). These variations may be due to differences in past pesticide and/or fertilizer use.

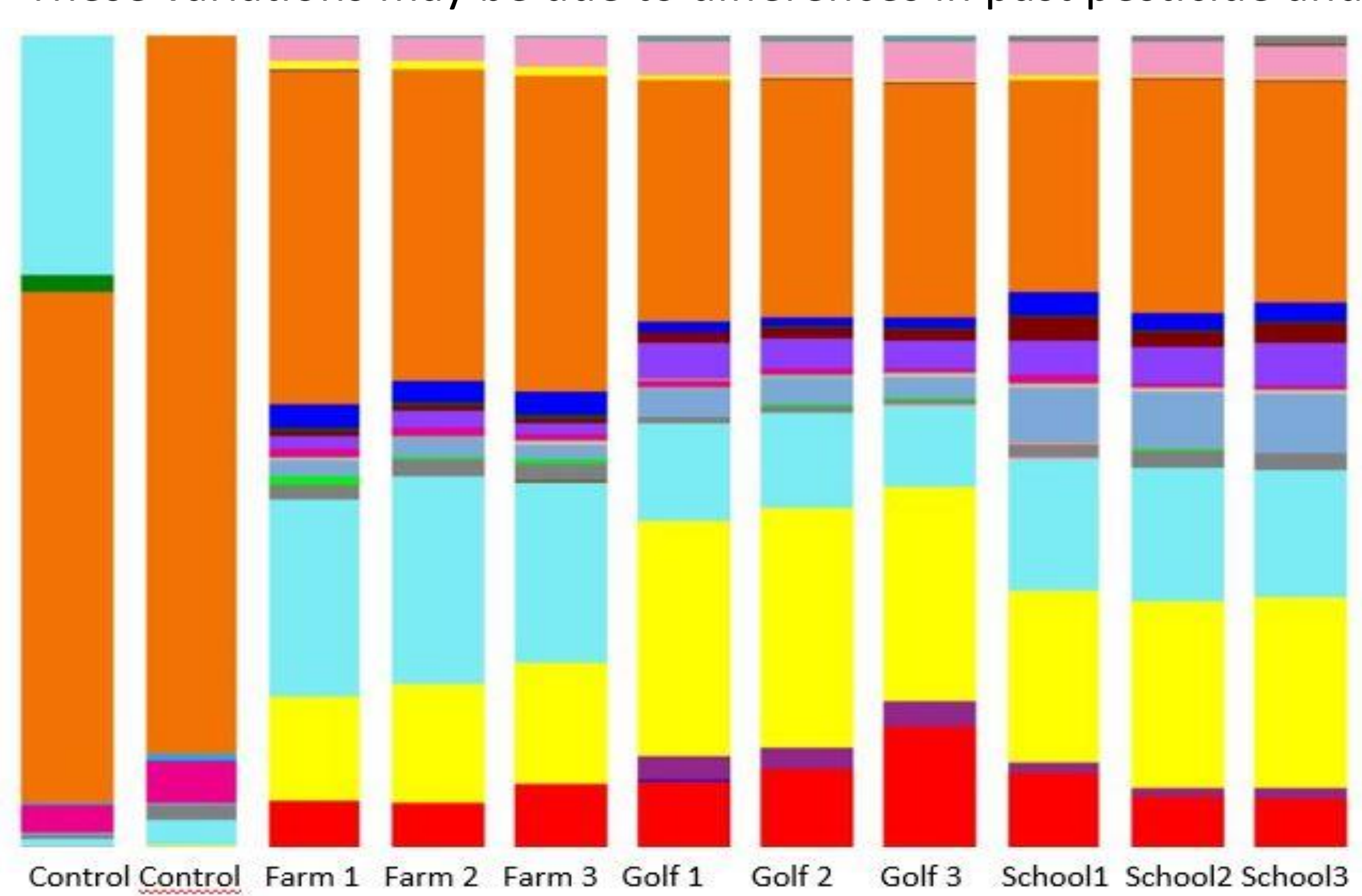

Figure 1: This figure shows the taxa plots generated for the triplicate samples of Farm Soil, Golf Course soil, and School Campus Soil. All the samples have acidobacteria , actinobacteria and proteobacteria.. Acidobacteria is the lowest in the farm. The data seem to have a consistent pattern.

Legend	Taxonomy	Total %	NCBN %	NCSP %	TT1 %	TT2 %	TT3 %	TT4 %	TT5 %	TT6 %	TT7 %	TT8 %	TT9 %
	Unassigned;Other	6.8%	0.2%	0.0%	6.0%	5.6%	8.0%	8.5%	9.8%	15.1%	9.4%	6.4%	6.2%
	k__Archaea;p__Crenarchaeota	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%	0.0%
	k__Archaea;p__Euryarchaeota	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__AD3	1.1%	0.0%	0.0%	0.1%	0.1%	0.1%	2.9%	2.5%	3.0%	1.1%	1.3%	1.2%
	k__Bacteria;p__Acidobacteria	17.7%	0.1%	0.5%	12.7%	14.5%	14.9%	29.0%	29.6%	26.3%	21.1%	22.9%	23.4%
	k__Bacteria;p__Actinobacteria	14.3%	1.0%	3.2%	24.2%	25.5%	22.0%	12.0%	11.5%	10.0%	16.2%	16.2%	15.7%
	k__Bacteria;p__Armatimonadetes	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	k__Bacteria;p__BHI80-139	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__BRC1	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%
	k__Bacteria;p__Bacteroidetes	1.5%	0.7%	1.8%	1.7%	1.9%	2.1%	0.6%	0.9%	0.7%	1.6%	2.1%	2.0%
	k__Bacteria;p__Chlamydiae	0.2%	0.0%	0.0%	0.9%	0.4%	0.6%	0.1%	0.0%	0.1%	0.1%	0.2%	0.1%
	k__Bacteria;p__Chlorobi	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%
	k__Bacteria;p__Chloroflexi	3.3%	0.1%	0.2%	1.9%	2.1%	1.9%	3.2%	3.2%	2.7%	7.0%	7.0%	7.2%
	k__Bacteria;p__Cyanobacteria	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.1%	0.2%	0.2%
	k__Bacteria;p__Elusimicrobia	0.2%	0.0%	0.0%	0.3%	0.3%	0.3%	0.2%	0.3%	0.3%	0.3%	0.4%	0.4%
	k__Bacteria;p__FBP	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__FCPU426	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__Fibrobacteres	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%
	k__Bacteria;p__Firmicutes	1.4%	3.4%	5.2%	1.1%	1.0%	0.7%	0.8%	0.8%	0.5%	0.9%	0.5%	0.4%
	k__Bacteria;p__Fusobacteria	0.1%	0.2%	0.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__GAL15	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__GN02	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__Gemmatimonadetes	2.8%	0.0%	0.0%	1.6%	2.0%	1.4%	4.5%	3.8%	3.4%	4.4%	4.3%	5.3%
	k__Bacteria;p__MVP-21	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__NKB19	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__Nitrospirae	1.2%	0.0%	0.1%	0.6%	0.6%	0.6%	1.3%	1.4%	1.3%	2.8%	1.9%	2.3%
	k__Bacteria;p__OD1	0.2%	0.0%	0.0%	0.5%	0.5%	0.5%	0.1%	0.1%	0.2%	0.2%	0.1%	0.2%
	k__Bacteria;p__OP11	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__OP3	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__Planctomycetes	1.8%	0.0%	0.0%	3.0%	2.6%	2.8%	1.1%	1.1%	1.4%	2.8%	2.2%	2.4%
	k__Bacteria;p__Proteobacteria	39.8%	62.9%	88.2%	40.9%	38.4%	38.8%	29.7%	29.1%	28.6%	26.0%	28.6%	27.0%
	k__Bacteria;p__Spirochaetes	0.2%	2.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__TM6	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.2%	0.2%
	k__Bacteria;p__TM7	0.4%	0.0%	0.0%	1.0%	0.9%	1.0%	0.3%	0.3%	0.3%	0.3%	0.4%	0.3%
	k__Bacteria;p__Tenericutes	2.7%	29.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%
	k__Bacteria;p__Verrucomicrobia	3.2%	0.0%	0.1%	3.0%	2.9%	3.7%	4.2%	4.2%	4.8%	4.2%	4.1%	3.9%
	k__Bacteria;p__WPS-2	0.2%	0.0%	0.0%	0.2%	0.2%	0.2%	0.6%	0.6%	0.6%	0.0%	0.0%	0.0%
	k__Bacteria;p__WS2	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__WS3	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.3%	0.1%	0.7%	0.8%	1.2%

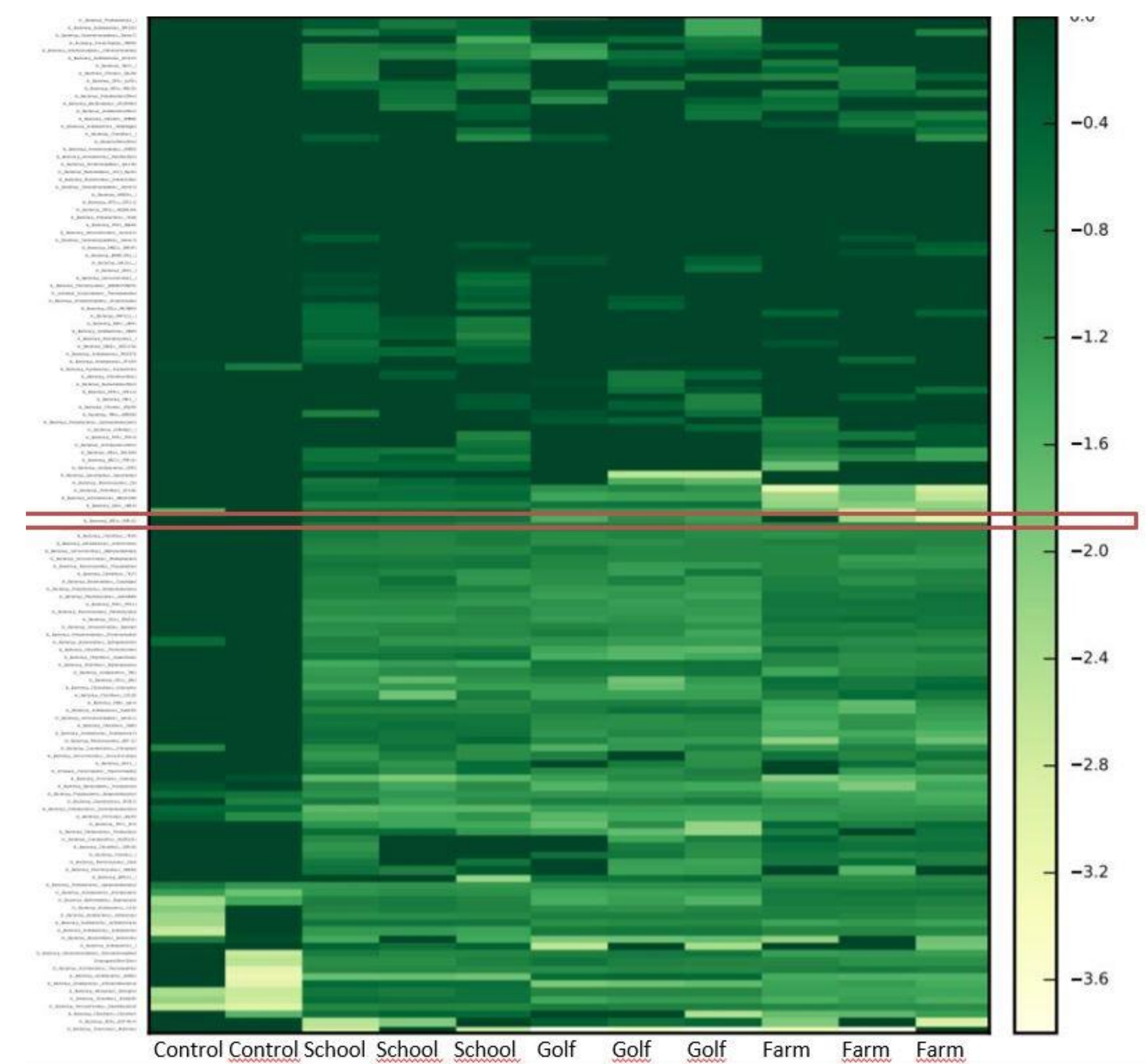

Figure 2: This plot represents the Level 3 (Class) phyla plot of the microbiome content of the triplicate Farm, Golf Course, and Longwood School Campus samples. Note the lack of armatimonadetes , plantomycetes, TM7, OD1 and clamydiae in the Farm samples, and the absence of WS2 and BRC1 bacteria in the Golf Course samples. Also note the high levels of Spirochaetes (boxed) in the Farm samples.

Discussion

The results of this study show differences in microbiome profiles between soil samples collected in different areas of the Longwood High School campus. These variations may be caused by different applications of pesticides and/or fertilizers. Interestingly, soil samples from a relatively small area (the campus and its borders) have been shown to exhibit different microbiome profiles, indicating that soil bacteria are sensitive to different environmental factors over time. While all samples contained proteobacteria, actinobacteria, and acidobacteria (Fig's 1,2) there were notable differences in levels of chloroflexi phylum bacteria and AD3 phylum bacteria (Fig 2). The findings have demonstrated a higher abundance of a recently discovered bacteria, AD3, in the farm soil, least in the golf course. Recent publication and cave studies have shown that AD3 is correlated with the increasing abundance of MgO which is commonly used in herbicides, fungicides, microbicides and pH adjustments. Farm soil also lacks common bacteria such as armatimonadetes , plantomycetes, TM7, OD1 and clamydiae (Fig 2) which could indicate a heavy use of pesticides. There is a complete lack of WS2 and BRC1 bacteria in the golf course (Fig 2). We are not sure of the significance of the absence of these bacteria in the golf course soil, but it may be associated with greater pesticide/fertilizer use.

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