Investigating the relationship between novel farming practices and zoonotic disease transmission at the wildlife/livestock interface: A case study at a commercial farm and conservancy in Beaufort West, South Africa

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Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where stated otherwise, by either reference or acknowledgment, the work presented is entirely my own.

Yameen Badrodien

28 April 2022

Abstract

In recent years, the intensification of agricultural activities in South Africa, has led to many of the sectors farmers adopting novel farming practices as a means of promoting economic productivity. One such practice, is the expansion of traditional livestock systems to include unconventional forms of stock such as wildlife. Despite the economic benefits of this approach, the threat of disease re/emergence remains a major obstacle, as both wild and domesticated animals are known reservoirs to a variety of disease-causing agents. Whilst many studies have directed their attention toward the surveillance of emerging pathogens within animal populations, less attention has been directed towards monitoring pathogens within environmental disease reservoirs (i.e., soil), which may be affected by these novel farming practices. This study sought to provide a longitudinal analysis of soil bacterial community composition at the Krommelboog Farm, a commercial livestock farm, and wildlife conservancy located in Beaufort West, South Africa. This investigation sought to evaluate the long-term impacts of animal integration on soil bacterial community composition – which dictates the propensity for soil borne disease transmission. Twelve soil samples were collected during 2017, during which time the farms animal populations remained segregated, and a further sixteen soil samples were collected during 2019, following their integration. DNA was extracted from samples, and the 16S rRNA gene (V3-V4 hypervariable region) was targeted for amplification. Purified amplicons were sequenced using high-throughput Next Generation Sequencing (NGS) technologies (Illumina MiSeq, 2013). DNA sequences generated by the aforementioned process were then subjected to *in silico* treatments using QIIME 1.9.1. Our elucidation of soil bacterial communities highlighted a shift in bacterial community structure following animal integration at the farm. *A*-diversity metrics (Shannon, Chao1 OTU and PD whole tree) indicated that integrated sites demonstrated significant differences in bacterial diversity, species richness and evenness when contrasted against samples collected under animal segregation. The observed shifts in bacterial community structures may be attributed to

differences in animal stocking rates (no. of animals per unit area), which has the potential to influence soil bacterial community composition by altering the physico-chemical properties of soils. The integration of animals at the Krommelboog Farm, increased the spatial range available for animal occupation, which has reduced the intensity of animal-induced soil disturbance, and ultimately promoted diversity among soil bacterial communities. Our investigation also led to the detection of 26 pathogenic bacteria. Detected bacteria demonstrated a greater diversity within integrated areas, but a lower overall contribution to the total bacterial community composition when contrasted against pathogens found within segregated areas. Despite the presence of pathogens across our sampled scenarios, these assemblages existed at densities too low to constitute a threat to either animal/human health or economic activities at the Krommelboog Farm.

Keywords: agriculture, livestock, wildlife, 16S rRNA, NGS, soil, bacteria, QIIME 1.9.1

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Introduction

1.1 Preamble

The role of agriculture, in an economic sense, is well documented in literature and accepted by scholars and economists alike (Machethe, 2004). While its integral role in global economics cannot be denied, we find that no consensus has been reached regarding its utility as an economic tool in developing nations (Machethe, 2004). As heavily debated as the aforementioned topic might be, one cannot ignore the fact that the vast majority of sub-Saharan Africa's population is directly or indirectly dependent on agriculture (Machethe, 2004; Diao, Hazell & Thurlow, 2010).

Considering the agricultural sectors significant contribution to the region's economy, it would be obvious that agriculture be considered as a key sector in the development of the region's economy (Diao, Hazell & Thurlow, 2010). If we consider the role that agriculture-based development has played in the alleviation of poverty and economic transformation of numerous Asian countries, one would assume that an agriculture-led approach would potentially yield similar results in sub-Saharan Africa (Diao, Hazell & Thurlow, 2010). Despite numerous attempts at implementing agriculture-led transformation in Sub-Saharan Africa, results have been unsuccessful.

The failure of many African countries to adopt the aforementioned approach is largely correlated with the regions inability to meet the minimum requirements for agricultural development, which is accurately reflected in sub-Saharan Africa's low agricultural productivity (Diao, Hazell & Thurlow, 2010). The principal barriers to the regions agricultural development may be ascribed to the low availability of both arable land and water resources (Ruttan, 2002). While soil erosion and degradation of soil have been identified as a serious hindrance to agricultural growth in developing and developed nations alike, the effects thereof are often amplified across semi-arid and arid areas (*viz*. sub-Saharan

Africa), where the availability of arable land has been significantly reduced and agricultural capacity severely compromised (Ruttan, 2004).

Matters are further exacerbated by the scarcity of water across the African continent, which has served as a serious constraint on the expansion of agricultural activities (Raskin et al., 1997; Seckler, Barker & Amarasinghe, 1999; Gleick, 2000). Moreover, Seckler, Barker & Umarasinghe (1999) believe that the limitations imposed by water scarcity across sub-Saharan Africa are only set to intensify over the next 15 years, which will see numerous sub-Saharan African countries experiencing either absolute or severe water scarcity. While these constraints are unlikely to affect global food production over the next half century, they do possess the potential to counter agricultural efforts in the world's poorest countries (Ruttan, 2004). It is anticipated that the inability to meet these requirements, together with the historical bias exhibited towards the sector, will invariably perpetuate the region's poor agricultural performance and productivity (Schiff & Valdés, 1992; Fan, Zhang & Rao, 2004; Timmer, 2005).

Despite the merit of an agricultural approach towards economic growth and development, the method is constrained by costs and barriers, which limit its success. If we consider the dynamics of the population-poverty-environment nexus as described by Malthus (1798), we realize that any measures taken towards mitigating the effects of food insecurity and economic instability are typically fulfilled at the expense of the environment. As agricultural activities are intensified, there is a greater propensity for natural environments to be subjected to anthropogenic pressure, which may lead to the loss of habitats and ultimately biological diversity (Daszak, Cunningham & Hyatt, 2001; Karesh et al., 2012, Meissner et al., 2013). More importantly, the interface at which anthropogenic transformation takes place creates the ideal scenario for the emergence and re-emergence of zoonotic diseases (Daszak, Cunningham & Hyatt, 2001; Karesh et al., 2012; Meissner et al., 2013).

1.2 Developing countries, disease and agriculture

The World Health Organization (WHO) defines zoonotic diseases as those diseases transmissible from animals to humans, resulting from the direct contact with animal products or environmental components, which serve as reservoirs for zoonotic disease-causing agents (WHO, 2009). It is believed that approximately three-quarters of emerging pathogens and at least two-thirds of human pathogens are zoonotic in their origin (Taylor, Latham & Mark, 2001; WHO, 2006; Jones et al., 2008). Further studies by Woolhouse & Gowtage-Sequeria, (2005), concluded that 13.0% of these pathogens are considered to be emerging and that up 70.0% of these emerging pathogens are considered to be of a zoonotic nature.

Karesh et al. (2012) and Meissner et al. (2013) highlighted the impact of these zoonotic diseases on human health and livelihood, as these diseases are globally implicated in over one billion cases of morbidity and at least one million cases of mortality every single year. Moreover, the impact and prevalence of these diseases has intensified in recent decades (Morse, 1995; McMichael, 2004; Harper & Armelagos, 2010). The expansion of trade and global travel, novel farming practices and the exponential growth of both livestock and human populations, have ultimately set the tone and tempo for disease emergence and associated pandemics (Morse, 1995; McMichael, 2004; Harper & Armelagos, 2010).

Recent data shows that the majority of recently emerged zoonotic diseases have their origins in wildlife, with an elevated risk of outbreaks occurring across equatorial regions across the globe (Jones et al., 2008). As a consequence of this, we find that developing countries suffer immense burdens to human health and even greater impacts on human livelihood when contrasted to that of developed countries (Maudlin, Eisler & Welburn, 2009). According to King, (2011), and Grace et al., (2017),

zoonotic diseases are more closely associated with impoverished communities, where they have been known to disproportionately affect these often-neglected populations. The situation is further aggravated by the fact that these areas are typically ill-equipped in terms of their ability to diagnose cases, often leading to the underreporting of diseases within these locales (Maudlin, Eisler & Welburn, 2009). Where underreporting is prevalent, the task of establishing the impact of zoonotic diseases becomes problematic, and more often than not, culminates in a poor understanding of disease dynamics in respective regions (Maudlin, Eisler & Welburn, 2009; King, 2011).

As is the case with many zoonotic diseases, we find that livestock have the capacity to serve as both amplifier and intermediate hosts, in which these pathogens may evolve and be transferred to humans indirectly via spill over, or directly through contact with wildlife or vectors (Childs, Richt & Mackenzie, 2007). Thus, the greatest challenge facing these underdeveloped countries lies in the occupational risks experienced by small livestock farmers, who are placed at greater risk of contracting zoonotic diseases such as bovine tuberculosis, anthrax and brucellosis, as farmers often find themselves in close contact with both livestock and the environments, that they occupy (King, 2011). The gravitas of this situation is realized when one considers the fact that developing nations comprise 700 million food insecure livestock farmers, and where livestock farming has been identified as a significant contributor to 70.0% of the worlds rural impoverished individuals (WHO, 2006).

Zoonotic diseases and the spread thereof in the modern world may be attributed to changes in human behaviour, which are closely associated with advances in both economic and technological development; the exponential growth of the global human population and the corresponding spatial expansion of agriculture (Morse, 1995; McMichael, 2004; Woolhouse & Gowtage-Sequeria, 2005).

Collectively speaking, the aforementioned factors have been implicated in the development of neoteric and intensive approaches towards agricultural development, the consequence of which is a marked increase in the interaction between humans, livestock, and wildlife (Morse, 1995; McMichael, 2004; Woolhouse & Gowtage-Sequeria, 2005, Tomley & Shirley, 2009). These factors have, in their own right, been identified as catalysts and drivers for countless cases of disease emergence the world over, many of which have had serious repercussions on both human health and livelihood (Morse, 1995; McMichael, 2004; Woolhouse & Gowtage-Sequeria, 2005). Evidently, the expansion of the human population, and the intensification of the aforementioned interactions, will in all probability drive future disease emergence/re-emergence, as mankind and its disease-causing pathogens proceed to evolve in unison (Tomley & Shirley, 2009).

In order to better understand the impact that anthropogenic activities have on disease emergence, the manner in which humans alter the environment as a means of intensifying agricultural productivity, needs to be taken into consideration. Firstly, anthropogenic activities are notorious for their role in ushering in environmental changes. Based on studies by Jones et al. (2013), the expansion of human settlements and agricultural areas into natural environments has the potential to result in the extension of ecotones (i.e., those areas which serve as a transitional zone between adjoining ecological ecosystems), facilitating the interaction between occupants of each of the respective habitats. Furthermore, Jones et al., (2013), suggests, that by increasing the interaction between species of adjacent environments, novel opportunities are created, in which spill over, adaptation, and genetic diversification of pathogens may be expedited.

The intensification of livestock production systems, on the other hand, facilitates disease transmission by elevating both population size and density, while lowering the total area utilized for production (Graham et al., 2008; Cutler, Fooks & Van der Poel, 2010; Drew, 2011). Moreover, intensive

agricultural production has a tendency to rely on antibiotic application as a means of promoting both animal growth and health, and lowering economic losses associated with disease (Gilchrist et al., 2006). As cost effective as this approach may be, it has been shown that this practice encourages the evolution of antibiotic resistance in zoonotic disease-causing pathogens (Gilchrist et al., 2006). Furthermore, the intensification of animal production generally involves high levels of translocation for animals across the area of production, which may further exacerbate the potential for pathogen transmission (Leibler et al., 2009).

The aforementioned information supports the fact that a movement towards more sustainable agricultural systems needs to take place, and that this movement is essential if global food requirements are to be met in the future. These systems should ultimately reduce the prevalence and impact of emerging infectious diseases; maximize agricultural throughputs; while simultaneously conserving the environment, biodiversity and human health. Central to this approach would be an improved understanding of disease dynamics, with a special emphasis placed on the acquisition of knowledge relating to the drivers of disease emergence.

1.3 The origins and evolution of game farming: A South African perspective

Over the past half-century, food systems the world over have undergone extensive change and development. Perhaps the most notable of which, as identified by Bengis, Kock & Fischer, (2002), has been the expansion of wildlife production, which has proven to be an effective tool in expediting both agricultural growth and profitability. Game farming itself, is a loose term that embraces a wide range of activities that focus on the exploitation of wildlife at varying intensities. For the purpose of this paper, the term "game farming" is taken to refer to "the management of free-living wild animals across large areas of private or communal land, where said animals are utilized for meat, by-products,

commercial hunting or ecotourism" (Bothma, 2002; Butler et al., 2005). Central to this method of farming is the introduction of diverse wildlife populations into areas that had previously, or continue to be used for, the farming of livestock (Bengis, Kock & Fischer, 2002).

As an agricultural practice, game farming in South Africa, is thought to have emerged during the 1960's – since which it has grown due to its ability to support a diverse array of intermediate revenue generation streams (Dekker & Van der Waal, 2000; Du Toit, 2007; Steyn, 2012). Following its emergence, game farming received formal recognition by the South African government in 1987, as an activity capable of contributing significantly to the agricultural sectors economic growth and development (Gouws, 2014). What had originated as an alternative means of animal production, often being practiced on land deemed unsuitable for livestock production, has evolved into a globally recognized, multidimensional industry, providing a diverse array of consumable and non-consumable products (Chardonnet et al., 2002; Lindsey, Roulet & Romañach, 2007; Chiyangwa, 2018, Taylor et al., 2020).

Since its inception during the 1960's, the total number of game farms in South Africa has grown to ~14, 000 farms, encompassing in excess of eight million hectares of land, a figure which continues to expand annually (Child et al., 2012; South African Yearbook, 2015-2016). This statement is supported by the findings of Cloete, Taljaard & Grove, (2007), who reported a 5.6% increase rate per annum, for land being utilized by the industry.

A study conducted by Van Hoving & Petronella, (2011), illustrated the distribution and intensity of wildlife production across South Africa (Table 1.1). Findings of this study revealed that the most extensive wildlife production units were located around the northern most reaches of the country, and included Limpopo, the Northern Cape and Northwest Provinces which collectively accounted for

75.24% or 8,542,540 hectares of all wildlife production units across South Africa (Table: 1.1) (Van Hoving & Petronella, 2011).

Table 1.1: A provincial overview of South Africa's wildlife production units (2011)

Province	**No. of wildlife production units**	**% of total wildlife production units**	**Total size (ha)**	**% Total area**	**Average unit size (ha)**
Freestate	180	3.56	147 743	1.43	821
Limpopo	2482	49.04	3 325 652	32.09	1340
Northwest	340	6.72	364 935	3.52	1073
Mpumulanga	205	4.05	276 016	2.66	1346
Guateng	72	1.42	82 076	0.79	1140
KwaZulu-Natal	90	1.78	168 841	1.63	1876
Eastern Cape	624	12.33	881 633	8.51	1413
Northern Cape	986	19.48	4 852 053	46.82	4921
Western Cape	82	1.62	265 205	2.56	3243
Total	**5061**	**100.00**	**10 364 154**	**100.00**	**2047**

Source: Van Hoving & Petronella (2011)

Studies by Bothma & Du Toit, (2016), estimated the game industry in South Africa to comprise a total of 18-20 million heads, a stark comparison to the 575, 000 heads of wildlife being farmed during the 1960's. Of the ~14, 000 South Africa game farms, 66.0% are farming exclusively with wildlife, while the remaining 34.0% are believed to be farming with a mixture of both domesticated livestock and wildlife (Child et al., 2012).

The magnitude of game farming in South Africa is well reflected in its fiscal contributions to the nation's economy. While one cannot deny the contribution of game farming to South Africa's GDP, the accuracy of these estimates remains questionable. Studies by Brink et al. (2011) have suggested that the industry is capable of generating up to 20 billion ZAR per annum, while studies by Bothma & Du Toit, (2016), reported a total contribution of 7.7 billion ZAR in 2010. Based on current growth trends registered by the industry in the past decade, the game industry shows considerable economic potential, and is likely to expand as it garners the attention of those that are able to fund the transition (Chiyangwa, 2018)

When confronted with the aforementioned information, one is inevitably prompted to question the appeal that game production has amongst South African farmers. While the benefits of game farming appear to be multitudinous, the most notable advantages of this method are closely associated with its practicality and profitability. Studies by Pollock & Litt, (1969), have indicated that game require far less provisions and routine maintenance when compared to that of domesticated livestock. The fact that wildlife tend to move in fragmented groups, means that they diminish resources at lower rates than herd animals such as cattle and sheep, a feature which inevitably allows them to be sustained over period of low water and food availability (Pollock & Litt, 1969). Furthermore, game animals tend to exhibit greater resilience against diseases, which unsurprisingly allows them to occupy a wider range of environments than their domesticated counterparts.

Walker, (1976), however, argued that the aforementioned benefits are ultimately counteracted by game farming's high capital investments, which include the establishment of infrastructure (e.g., fences and waterholes) as well as the exorbitant costs associated with game acquisition.

Despite the validity of either of these arguments, studies by Steyn, (2013), highlighted the stark differences in earning potential between the aforementioned farming approaches. The findings of this study showed that a typical game farm of average profitability was capable of generating approximately 2,640.00 ZAR per hectare per annum, whereas livestock farms performed considerably poorer, generating a mere 960.00 ZAR per hectare per annum. Based on these findings, the appeal of game farming among South African farmers could very well be governed by the profitability associated with wildlife production.

Despite the economic viability of the aforementioned approach, the sustainability of the practice has, in recent years, been brought under scrutiny (Skinner, 1970; Morse, 1995; McMichael, 2004; Woolhouse & Gowtage-Sequeria, 2005, Tomley & Shirley, 2009). The fact that wildlife populations are known to harbour zoonotic disease-causing pathogens, many of which could incur serious health and economic repercussions if left unaddressed, remains one of the strongest arguments against the adoption of this method (Morse, 1995; McMichael, 2004; Woolhouse & Gowtage-Sequeria, 2005; Tomley & Shirley, 2009). Having considered the aforementioned, it is evident that the threat of disease transmission within and between wild animal populations exists, however, Bester & Penzhorn, (2002), have stated that the potential for disease transmission may be further elevated in scenarios where wild and domestic populations have been integrated.

The rationale behind this statement is supported by findings by Drew, (2011), who highlighted the impact that animal diversity and population density have on disease transmission and adaptation.

Generally speaking, intensive livestock farms are characterized as having high-density populations, with low genetic diversity across a limited spatial environment, factors which have been demonstrated to drive both disease transmission and pathogen adaptation (Drew, 2011).

Epidemiological modelling performed by Springbett et al., (2003), evaluated the relationship between the probability and intensity of outbreaks against the genetic diversity of animals being farmed, the outcome of which, indicated that populations exhibiting low genetic diversity (*viz*. high intensity livestock animal production) demonstrated a higher probability for major disease epidemics, whereas populations exhibiting high genetic diversity (*viz*. pure wildlife production or mixed animal farming) exhibited a greater probability for minor disease epidemics. These findings were echoed by Keesing et al., (2010), in which the impacts of biodiversity on the emergence and transmission of infectious diseases were assessed. Taking the aforementioned into consideration, it would then follow that: the exposure of livestock to pathogens present in wildlife populations may account for the initial transmission of disease, however, the extent of outbreaks appears to be governed by characteristics inherent to intensive livestock production (i.e., low genetic diversity amongst livestock).

While the dynamics of disease emergence/re-emergence and proliferation are closely governed by the underlying genetics of animals being farmed, zoonosis by its very nature is indiscriminate, leaving both wild and domesticated populations susceptible to outbreaks (Bengis, Kock & Fischer, 2002; Springbett et al., 2003). Within the game industry, we find that emergence itself, is aggravated by certain practices (*viz*. animal translocation) (Daszak, Cunningham & Hyatt, 2000). According to studies by Daszak, Cunningham & Hyatt, (2000), the translocation of animals gives rise to reverse spill over ('spillback'), in which sympatric livestock populations are affected. In contrast, wildlife populations that have been integrated into traditional livestock systems, risk being exposed to cycling zoonotic pathogens present within livestock populations (Bengis, Kock & Fischer, 2002).

Despite the inherent risks associated with wildlife production, South African farmers have continued to abandon traditional livestock farming in favour of either exclusive or mixed wildlife production, a shift that has invariably complicated the roles of those involved in disease control and rangeland management (Bengis, Kock & Fischer, 2002; Bester & Penzhorn, 2002).

As the burden of zoonoses continues to be realised, the topic of disease, be it emerging or re-emerging, has become a common agenda item across agricultural sectors the world over (Brahmbhatt, 2005; Jones et al., 2008). In an effort to mitigate the economic and health repercussions associated with zoonotic diseases, a great deal of effort has been invested into targeted disease surveillance, which has the potential to improve efforts directed at mitigating the burden of zoonoses (Karesh et al., 2005; Vrbova et al., 2010). While efforts at disease surveillance have, in recent years, intensified, recent approaches towards disease control and surveillance have for the most part, targeted specific diseases and tend to follow a reactive framework (Childs & Gordon, 2009). The adoption of this approach is largely governed by its practicality, which more often than not, relies on the identification of human cases as the primary means of surveillance (Childs et al., 1998; Childs & Gordon, 2009).

1.4 Zoonotic disease management: Surveillance, detection and control

Anthony et al., (2013), and Lipkin, (2013), have suggested a proactive approach to disease management, which would require the investigation and discovery of pathogens in wildlife as a means of mitigating the burden of zoonoses in the realm of mixed animal production. However, efforts at a proactive approach to disease management are currently constrained by inadequate disease investigation techniques as well as an incomplete understanding of disease transmission dynamics (Jones et al., 2008; Childs & Gordon, 2009; Anthony et al., 2013). Moreover, the routine surveillance of natural hosts often requires multidisciplinary teams of epidemiologists, mammologists, physicians

and ecologists, rendering the approach impractical in most instances (Childs et al., 1998). Finally, one needs to consider feasibility of natural host surveillance, as most zoonoses are unresponsive to eradication efforts, except in those instances where animal reservoirs are targeted for vaccination (Childs et al., 1998).

In an effort to bridge the gap that exists for zoonotic disease surveillance and management, efforts have recently shifted their focus toward factors associated with disease emergence (i.e., environmental pathways such as soil and water), thereby ensuring that resources are pooled in areas where disease emergence is more likely to take place (Morse, 1995; Taylor, Latham & Mark, 2001; Woolhouse & Gowtage-Sequeria, 2005; Jones et al., 2008).

Previously, the surveillance of environmental pathogens depended entirely on methods employing either culture or BLAST (Basic Local Alignment Search Tool) polymerase chain reaction approaches or a combination of both (Law et al., 2015; Rudkjøbing et al., 2016). While either of the aforementioned approaches are indispensable in the field of pathogen detection, the utility of these methods are less suited to broad-scale bacterial investigations of environmental samples (e.g., soil, water and faecal matter) (Suzuki & Giovannoni, 1996; von Wintzingerode et al., 1997; Polz & Cavanaugh, 1998). While these methods remain indispensable in the field of pathogen detection, either method is constrained by the complex nature of the samples being analysed.

The issues surrounding culture-based approaches are thoroughly highlighted by Hasman, (2014), who regards the approach as being time and resource consuming, especially in those instances where bacteria being isolated exhibit slow growth or fastidious tendencies. In addition to this, the heterogeneous nature of soil has been known to complicate soil culture efforts, as bacterial assemblages are often aggregated unevenly within the medium, thereby reducing the veracity of any

deductions made regarding bacterial density and diversity (Wall & Virginia, 1999). According to Kirk et al., (2004), the inaccuracy of the aforementioned approach is underpinned by the presence of numerous microhabitats present within a body of soil, each of which are capable of supporting a unique suite of bacterial assemblages. Furthermore, soil cultures may be constrained the inability of certain bacterial types to be cultured. Studies by Borneman et al., (1996), and Giller et al., (1997), have suggested that up to 99.0% of soil bacteria observed using microscopy are incapable of being cultured using standard culture techniques. It follows that the 1.0% of culturable bacteria may not be an accurate representation of the entire bacterial community present within soil (Rondon et al., 1999).

Other traditional methods of investigation such as BLAST PCR exhibit a number of inherent limitations (von Wintzingerode et al., 1997). The most notable of these limitations include yield biases in product to template ratios of targeted sequences, which may arise during the amplification process (Suzuki & Giovannoni, 1996; Polz & Cavanaugh, 1998). The implications of the aforementioned, may also promote inaccuracies pertaining to bacterial density and diversity of soil samples.

While either of their aforementioned techniques are considered to be gold standards in the field of bacterial investigation, the inaccuracies associated with the employment of these techniques for soil microbiome investigation are innumerable.

1.5 Zoonotic disease investigation methods: Technologies from past to present

In an effort to bridge the gap that has existed for bacterial community investigation, novel molecular methods have, in the past 30 years, been explored and developed. The majority of these methods include the employment of genomic analysis of environmental samples, which has since become an essential methodology in unravelling evolutionary history and clarifying the complexities associated

with ecological and functional biodiversity (Shokralla et al., 2012). Perhaps the most appealing factor associated with the aforementioned method includes its ability to forego laboratory cultivation and/or sample isolation, circumventing the challenges inherent to traditional forms of investigation (Shokralla et al., 2012). Typical environmental samples include, water, soil, sediment, gut contents as well as faecal matter, all of which may be utilized in the mapping a variety of bacterial microbiomes (Shokralla et al., 2012; Jansson & Baker, 2016).

The first generation of genomic sequencing methods (i.e., automated Sanger sequencing) emerged during the late 19th century, from which point it slowly gained traction and proceeded to become the foremost sequencing technology for the next 20 years (Metzker, 2010). Through the advancement of technologies associated with the method, Sanger-led approaches inevitably gave rise to a variety of comprehensive biosystematics endeavours, exhibiting a broad range of technical applications (e.g., Barcode of Life Initiative, Hajibabaei et al., 2007). Throughout its heyday, Sanger-led sequencing continued to demonstrate its indispensability as a molecular tool, garnering an innumerable number of prodigious accolades; the most notable of which included the finalization of the only finished grade human genome sequence - a monumental achievement of the era (International Human genome Consortium, 2004).

Central to the aforementioned method of genomic analysis is the employment of standardized genomic regions, or DNA barcodes, that are capable of generating extensive libraries of information, which could then be utilized in the process of identifying uncharacterized target specimens (Shokralla et al., 2012). In the case of bacterial investigation, the primary DNA barcode employed for the task of identification, is the 16S ribosomal RNA gene, which has become a common housekeeping tool in the field of bacterial bioinformatics (Sogin et al., 2006; Flanagan et al., 2007, Janda & Abbott, 2007; Sabat et al., 2017). The advantages of this gene, as a DNA barcode, is underpinned by three key features:

Firstly, the gene is ubiquitous in all bacteria, where it commonly exists as operons or multi-gene families; secondly, the function of the gene is heavily conserved within bacteria, suggesting that arbitrary sequence deviations may be regarded as a more precise measurement of evolutionary time; and finally, the size of the gene (1500bp) lends itself very well to bioinformatics applications (Patel, 2001; Janda & Abbott, 2007). The utility of the 16S rRNA gene, more specifically the universal primer base of the V3-V4 hyper-variable region, received further corroboration by studies by Takahashi et al., (2014), the findings of which emphasized the regions DNA recovering capabilities. The aforementioned study, during which these primers had specifically been developed, demonstrated the methodology's ability to detect and recover up to 94.6% of archaea and 98.0% of bacterial derived DNA (Takahashi et al., 2014). Moreover, the region exhibited an improved ability to detect atypical bacterial strains, and overall, a greater number of bacterial species when compared to the universal bacterial primer (Takahashi et al., 2014; Zhang et al., 2017).

The technical abilities of Sanger-led sequencing technologies in the field of bacterial investigation however, have been constrained by the protocols inability to sequence specimens en masse, rendering the application unsuitable for broad-scale bacterial investigations, where the DNA of hundreds or even thousands of bacterial taxa are being dealt with (Shokoralla et al., 2012). While one cannot deny the contributions made by conventional sequencing in the generation of extensive DNA barcoding libraries, the number of bacterial individuals present in environmental samples needed for bacterial microbiome investigations falls beyond the realm of the methods sequencing capabilities (Hajibabaei., 2011).

In an attempt to overcome the aforementioned issues, extensive amounts of energy, time and resources have, over the past 20 years, been invested into improved technologies (i.e., Next Generation technologies or NGS). The selling point of NGS technologies as an improved alternative to

conventional sequencing (*viz.* automated Sanger sequencing) may be attributed to the approach's ability to recover DNA sequences from multiple taxa present within environmental samples, a feat that hinges upon the methods ability to read DNA several templates in parallel (Drancourt et al., 2000; Woo et al., 2003; Mignard & Flandrois, 2006; Sogin et al., 2006; Shokoralla et al., 2012).

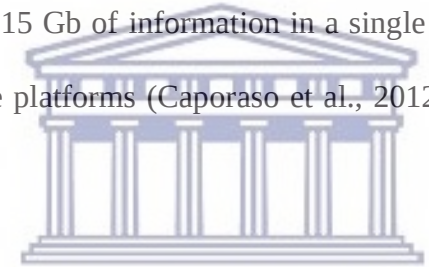
Data generated through NGS sequencing efforts have been utilized in a broad array of applications, which includes: the comparison of microbiota present in healthy and diseased organisms (e.g., Andersson et al., 2008; Zhang et al., 2009); the investigation of archaic DNA (Haile et al., 2009; Sonstebø et al., 2010; Boessenkool et al., 2012); the evaluation of ecosystem health as a function of bacterial diversity (Hajibabaei., 2011) and dietary analysis from gut and faecal derived DNA fragments (Deagle et al., 2009).

With regards to investigating the composition of bacterial communities present in environmental specimens, the most appealing features of NGS include its ability to provide genus and species level identification for isolates that are characterized by "low likelihood strains" or "adequate" identification and classifications based on commercially available protocols; strains that fail to fit any standard biochemical profiles or are known to include taxa that are less frequently associated with human pathogenesis (Drancourt et al., 2000; Woo et al., 2003; Fontana et al., 2005; Mignard & Flandrois, 2006). Moreover, the comparison of obtained sequences to an ever-expanding, standardized reference library, bacterial assemblages isolated from environmental samples may be identified with a high level of confidence (Shokralla et al., 2012).

To date, there exists a variety of NGS sequencing platforms, each of which may be employed in bacterial microbiome investigation. These platforms include: Ion Torrent (Salipante et al., 2014), 545 pyro-sequencing (Margulies et al., 2005) and Illumina (Caporaso et al., 2011, Caporaso et al., 2012,

Degnan & Ochman, 2012, Wu et al., 2015), all of which possess practical utility in molecular investigation. Despite the capabilities of each of the aforementioned platforms, the Illumina MiSeq system has gone on to establish itself as the dominant sequencing platform within the industry (Caporaso et al., 2011; Caporaso et al., 2012; Degnan & Ochman, 2012; Wu et al., 2015).

Illumina's popularity, according to Caporaso et al., 2012, Gibson et al., 2014 and Wu et al., 2015), is largely attributed its cost-effectiveness when contrasted against other commercially available sequencing platforms. The low costs associated with the platform has inevitably resulted in the democratization of sequencing within the scientific community (Tringe & Hugenholtz, 2008), but more specifically amongst those involved in microbiome investigation. Moreover, Illumina's popularity is also underpinned by its large throughputs, with the platform capable of generating enormous amounts of data (up to 15 Gb of information in a single run) and lower error return rates when compared to other available platforms (Caporaso et al., 2012; Gibson et al., 2014; Wu et al., 2015).



The evolution of NGS together in conjunction with advances in computational methods, have facilitated an improved ability to infer bacterial diversity measures across space and time, through the clustering and annotation of DNA sequences using a combination of phylogenetic and assignment techniques (Hajibabaei et al., 2011). According to Shokralla et al., (2012), ecological research in recent years has exhibited an affinity towards the employment of high volumes of sequence data, this is well demonstrated in the recently observed expansion of studies employing NGS platforms, and represents a paradigm shift within the field. It therefore comes as no surprise, that the arrival of NGS technologies has in certain respects, been likened to the early stages of PCR, where the only limitation was one's imagination.

Considering the aforementioned information regarding currently available technical approaches, we see it fit that NGS technology, targeting the V3-V4 hyper-variable region of the 16S rRNA gene of bacterial assemblages within environmental samples, be employed as a means of addressing the questions posed by this study.

1.6 Study area and land management

The study area for this investigation was located at The Krommelboog Farm (-32.018, 22.862), a predator friendly commercial livestock farm and wildlife conservancy located 60 km from the rural Karoo town of Beaufort West, in the Western Cape Province of South Africa (Fig 1.1). The farm encompasses a total of 22,000 hectares, and is managed by the Landmark Foundation (LMF) for the purpose of both livestock and wildlife production and the conservation of biological diversity.

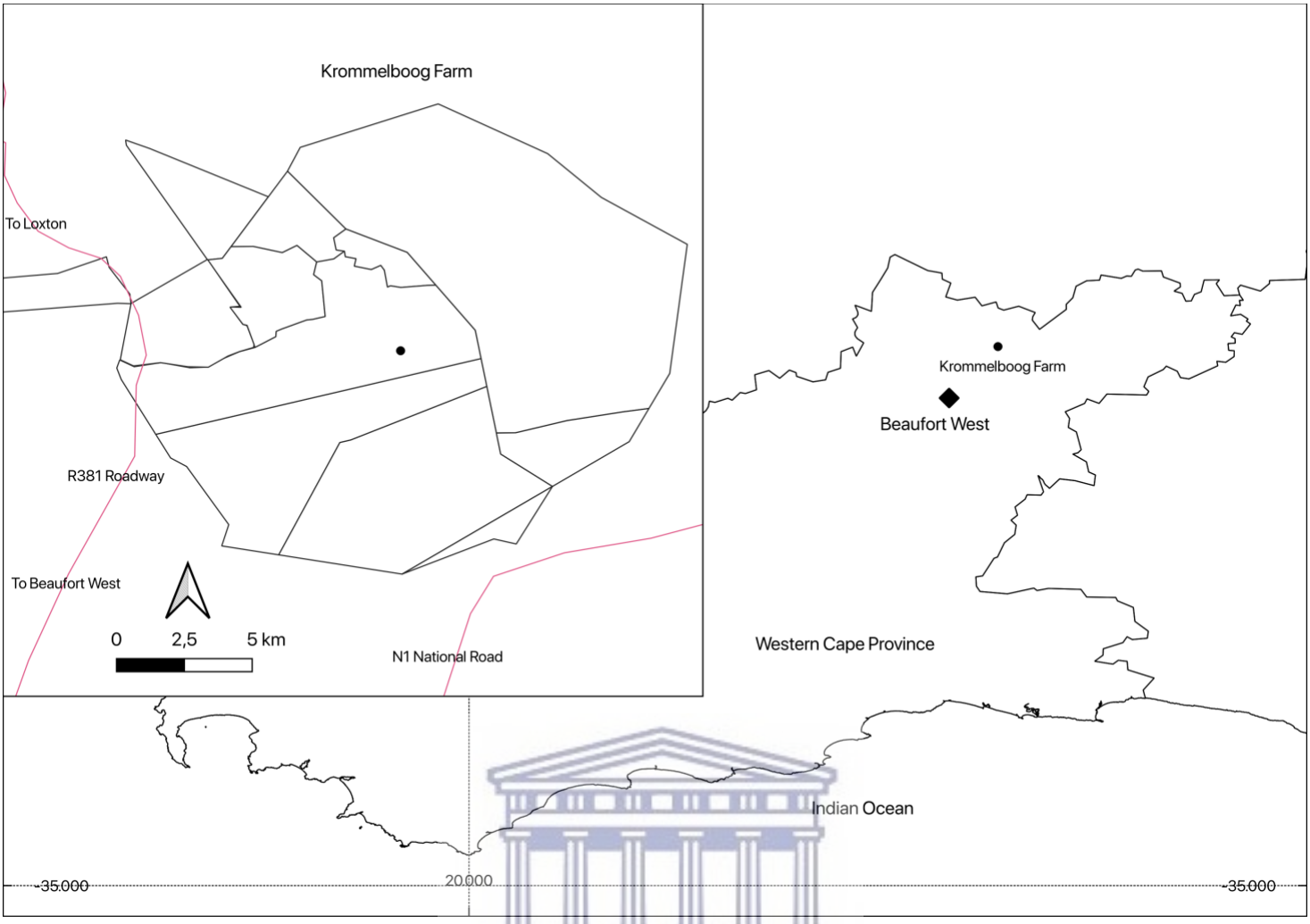


Figure 1.1: A graphical representation of the Krommelboog Farm, located in the Karoo region of the Western Cape Province, South Africa.

The Landmark Foundation

The LMF has spearheaded a wide variety of scientific studies across a multitude of locations within South Africa. These studies are focused primarily around species conservation, the expansion of protected areas, tourism development, expansion of local economies, the development of renewable sources of energy, waste recycling and the conservation of water and wetland areas. The foundation itself has partnered itself with a broad range of individuals, including but not limited to communities and their members, government sector agencies, private individuals and civil society groupings. The

activities of the LMF within the Karoo are centred on livestock production, wildlife production and biodiversity conservation. The LMF firmly advocates for sustainable agricultural practices, the most notable of which includes non-lethal predator management across of its project areas. The rationale behind this approach is driven by the fact that less than 20.0% of South Africa enjoys formal protected status recognition, and that this figure compromises efforts at biodiversity protection as well as the functioning of ecological systems. Given the fact that private livestock production units constitute such a large portion of South Africa's land cover, the most effective means of bolstering conservation efforts would to require us to focus our attention towards practices employed at these farms, ultimately allowing for the development and growth of sustainable food systems which promote the conservation of indigenous wildlife.

Rainfall

The region within which our study area is located experiences an average rainfall of 239 mm (South African Weather Bureau, unpublished data, 2017), which peaks during the summer season (October-April). Occasional rainfall and snowfall have been reported to take place during the winter months. During the period of sample collection, The Krommelboog farm experienced a total of 294 mm of rainfall, which was recorded on site for the period of January 2017 through to January 2018.

Geology and Soils

Geologically speaking, the farm is located on the Karoo Supergroup, which includes the Ecca Group, Dwyka Formation and the Beaufort Group (Turner, 1981). The Beaufort Group overlies the Ecca Group, and consists primarily of sandstone and mudstone (Turner, 1981). The Beaufort Group subdivides into the upper Teekloof Formation and the lower Abrahamskloof Formation, either of which are include sandstone, mudstone and thin cherty beds (Turner, 1981) The mudstone and sandstone layers within the aforementioned formations are indicative of floodplain and river channel

deposits respectively, whereas the presence of cherty beds is originally ascribed to ashfall tufts, and more recently, may have occurred via alluvial deposits (Turner, 1981). Within middle and upper plateaus, igneous dolerite intrusions are extensive (Walker & Poldervaart, 1949). Parent material sourced from slopes across the region are known to comprise siltstone, mudstone and sandstone along with dolerite intrusions accompanied by the presence of either Glenrosa or Mispah soil types (Walker & Poldervaart, 1949).

Topography

In a topographical sense, the farm is situated along the Nuweveld Mountain range, an area that is largely undulating, and variable in altitude. The plateaus in the area extend to heights of 1290 m with a few taller hills extending up to 1430 m in height, while the valley floors occur at an average height of 1150 m above sea level.

Vegetation

Two broad vegetation types, namely, the Upper Karoo Hardeveld and the Eastern Upper Karoo karroid, characterize the Krommelboog Farm. The former vegetation type is prevalent on steep hilly topographical areas across the farm, that are typically dotted with large boulders, and tend to comprise sparse dwarf Karoo scrub, and includes a number of drought tolerant grasses (Mucina et al., 2006). The latter vegetation type is prevalent across flat or gradually sloping landscapes which constitutes the remainder of the farm's topography, and includes a higher proportion of grasses, interspersed with low lying shrubs (Mucina et al., 2006). The xerophytic vegetation that characterizes the region, comprises mesembryanthemums, aloes, stapelias, euphorbias, and desert ephemerals, which are typically spaced 50 cm or further apart from one another, and tends to become sparser as one moves northward (Potgieter & Du Plessis, 1972).

Animal species

The Krommelboog farm is inhabited by a diverse array of animals that include conventional forms of livestock and wildlife. Traditional livestock at the farm encompasses 1100 head of Dorper and Damara sheep and 500 head of cattle, which are cared for by human shepherds, housed overnight in temporary kraal facilities and follow a planned grazing system. In terms of the farms wildlife assemblages, the farm boasts a range of ungulates including: springbok *Antidorcas marsupialis*), common duiker (*Sylvicarpa grimmia*), eland (*Taurotragus oryx*), blesbok (*Damaliscus pygargus phillipsi*), gemsbok (*Oryx gazelle*), Kudu (*Tragelaphus stepciceros*), plains zebra (*Equus quagga*) and red hartebeest (*Alcelphus buselaphus*), collectively contributing to a wildlife population of approximately 380 heads. In addition to the aforementioned, small populations of predatory cats also occupy the farm. These include the cape leopard (*Panthera pardus pardus*) and caracal (*Caracal caracal*).

1.7 Study Aim

The aim of this study is to map the bacterial communities present within soil and faecal samples across the various sample sites within the study area through the employment and application of NGS technologies. This process would allow for the delineation of the bacterial community structures (γ-diversity), and the quantification and the identification of core taxa at various taxonomic rankings. Further, we will attempt to elucidate, quantify and contrast the bacterial communities of sampled scenarios (contact, livestock and wildlife areas), and establish any differences in diversity, species richness and evenness, through the application of appropriate α-diversity analyses. In addition to this, the inter-scenario community composition will be assessed through the consultation of the scenario specific OTU's generated by our analysis. In an effort to establish the threat of pathogenicity at the Krommelboog Farm, collected samples will be screened for any pathogenic strains of bacteria, through the employment of user generated OTU tables and taxa summary reports provided by Illumina. The

information derived from these efforts would allow us the opportunity to contrast the proportion of identified pathogens against the total number of OTU's detected, while simultaneously highlighting any threats they may have on human/animal health and economic activities at the farm. Finally, with the aid of the data generated by our analyses, we intend to garner an improved understanding of the impact of animal integration on the diversity of soil bacterial communities at the Krommelboog Farm

1.7.1 Study Questions

1. What is the bacterial community composition (γ-diversity) of soils sourced from the various scenarios at the Krommelboog Farm?
2. How do our sampled scenarios vary in terms of phylum-level bacterial community composition (α-diversity)? Are there any observed differences in OTU abundance, diversity, species richness and evenness?
3. Are there any pathogenic strains of bacteria present at the Krommelboog Farm? In which of our sampling areas were these pathogenic strains detected? What are the contributions of these pathogens the total bacterial community? Do these bacteria have the potential to incur health or economic losses at the farm?
4. What deductions can be made regarding the partial integration of wild and domestic animal populations and the impact thereof on soil bacterial diversity, and pathogen prevalence at the Krommelboog Farm?

Chapter 2

Investigating the bacterial communities present amongst livestock and wildlife populations at The Krommelboog farm, Beaufort West, South Africa, and the interfaces that connect them

2.1 Introduction

The "wildlife/livestock interface", is a term that has, in the past 30 years, drawn a significant amount of attention from the global agricultural community (Bengis, Kock & Fischer, 2002; Osofsky & Cleaveland, 2008; Vicente, Vercauteren & Gortázar, 2021). The term has the ability to elicit a variety of images, ranging from the interaction of domestic and wild avian populations in China, through to scenes of livestock operations encroaching the natural habitats of wildlife on the dusty plains of Africa. In recent years, the outputs of numerous studies have advocated for the departure from traditional livestock farming, in favour of either wildlife farming, or integrated farming, especially amongst Africa's rural communities (Tomlinson et al., 2002; Chaminuka, 2013; Vetter, 2013). The promulgation of these campaigns is underpinned by a number of factors. Firstly, the profitability of wildlife production or mixed animal farming exceeds that of livestock farming (Tomlinson et al., 2002), a claim that is well evidenced by the economic outputs generated by this practice across a number of African countries (Behr & Groenewald, 1990; Cumming & Bond, 1992; Chardonnet et al., 2002). Secondly, the method is considered to be an effective conservation tool, which allows for the sustainable utilization of resources (Kock, 2005), and is capable of facilitating conservation efforts beyond the protected areas of a country (Munthali, 2007).

Despite the popularity gained by the aforementioned approach, the "wildlife/livestock interface" exhibits a dichotomy of impacts relating to biosecurity, economics, livestock health and production, all of which have become pertinent points of consideration for those involved in rangeland

management. To assess the integration of wildlife and livestock in terms of the aforementioned factors is beyond the scope of this study, instead, it directs its attention towards investigating the health and economic implications of animal integration at the wildlife/livestock interface.

The major concerns regarding mixed animal farming are tied into cases of epidemics or chronic disease problems as a consequence of disease introduction, which many have attributed to the coexistence of wildlife and livestock populations (Kock, 2005). Contrary to this statement, Bourn & Blench (1999) have advocated that disease transmission comes as consequence of a poor understanding of disease dynamics, rather than animal-borne diseases themselves. With that being said, disease transmission at the interface occurs bi-directionally, where there exists an equal potential for disease transmission from wildlife to livestock, and from livestock to wildlife (Bengis, Kock & Fischer, 2002). In general, disease surveillance on the livestock side of the interface rests primarily upon local veterinary authorities, depending on the country in question (Bengis, Kock & Fischer, 2002). In those instances where diseases have emerged or re-emerged, authorities have attempted to mitigate the spread through the implementation of activities such as: farm inspections, passive reporting, abattoir surveillance, and serological methods that often conducted in conjunction with other standard disease detection protocols (Bengis, Kock & Fischer, 2002; Heuvelink et al., 2007; Cameron, 2012; Bellini, Rutili & Guberti, 2016). In contrast to this, we find that disease monitoring and surveillance on the wildlife side of the interface tends to be less structured and therefore less accurate and successful. The difficulty associated with wildlife disease investigation is underpinned by unclear legal frameworks, which offer poor guidelines regarding the responsibility of disease surveillance and reporting (Bengis, Kock & Fischer, 2002). In addition to this, the majority of the aforementioned disease surveillance strategies are focused primarily on indigenous or exotic diseases, while emerging or re-emerging diseases are often overlooked (Bengis, Kock & Fischer, 2002; Kock & Heuer, 2019).

The Krommelboog Farm offered us a unique opportunity to investigate potential resource conflicts that may arise between wildlife and livestock at the wildlife/livestock interface. The farm is distinguished from other animal production units in the area due to its avantgarde approach towards rangeland management, the most notable of which include a "fence free' method of animal production. The decision to implement this practice is underpinned by the approach's ability to unify agricultural activities at the farm, while allowing for synchronization of these activities with local biodiversity conservation efforts. Given the fact that efforts at integrating the aforementioned animal populations are still underway (i.e., fence removal), the farm is currently characterized by two major scenario types. The first of which are non-contact scenarios, which reference those areas of the farm that are still subject to the presence of fences, and are currently populated exclusively by either wildlife or livestock. The second scenario type includes the "wildlife/livestock interface", or those areas where the integration of wildlife and livestock populations are already underway. Here we investigate the bacterial communities present within contact and non-contact scenarios at the Krommelboog Farm in an attempt to:

1. Delineate the bacterial community composition (γ-diversity) within soil and faecal samples collected at the Krommelboog Farm.
2. Elucidate and quantify the bacterial communities within sampled areas and contrast them against one another (α-diversity), while highlighting core contributing phyla and patterns in bacterial community structure.
3. Evaluate the intra-scenario bacterial community composition (β-diversity)
4. Screen the environment for pathogenic bacteria, that may incur health or economic losses at the Krommelboog Farm.

2.2 Materials and methods

2.2.1 Sample Collection

A total of twelve samples were collected in the form of either soil or dung (Table: 2.1), within non-contact (livestock/wildlife) and contact scenarios at Krommelboog Farm (Fig. 2.1). The rationale behind the collection of faecal matter in conjunction with soil samples, was motivated by the fact that soil samples alone would not be an accurate reflection of the area's bacterial community. While it is true that all bacteria present in faecal samples have the opportunity to assimilate with the environment (i.e., soil), many bacterial strains are host dependant, and are therefore less likely to persist for extended periods of time in the external environment (i.e., soil) following their departure from the host organism. In addition to this, special care was taken to ensure that all samples collected contained a high-moisture content, thereby improving the bacterial load for each of the sample.

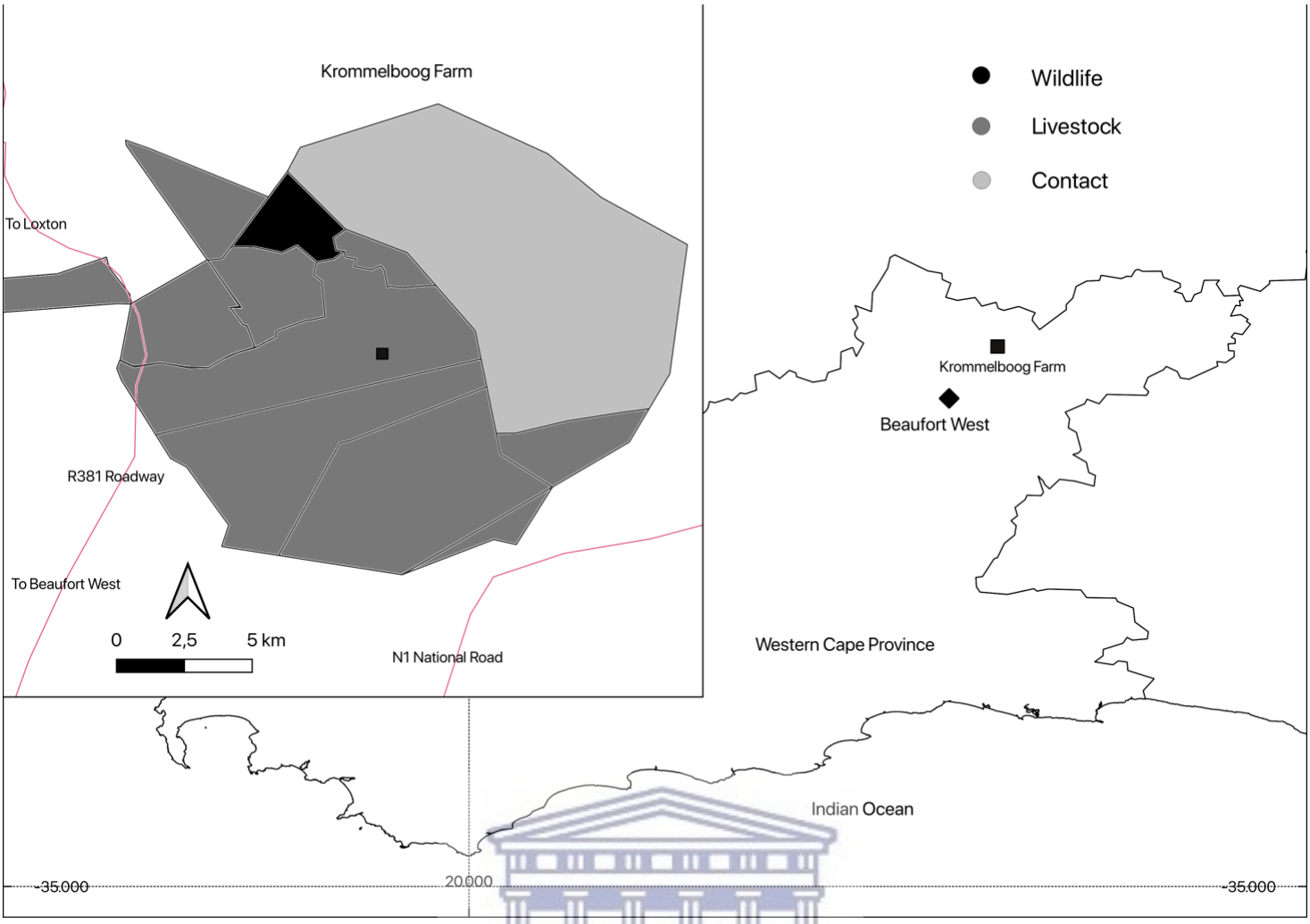


Figure 2.1: A map depicting the various scenario types (contact vs. non-contact) in which samples were collected at the Krommelboog Farm, Beaufort West South Africa.

Of the twelve samples collected, six samples were derived from livestock areas (non-contact), comprising a total three soil samples and three dung samples. Three samples were collected within the farms' wildlife areas (non-contact), comprising four dung samples and one soil sample. The remaining two samples were collected at the "wildlife/livestock interface" (contact), in the form of dung (Table: 2.1). It should be noted that all sampling sites were located at a distance of no less than two kilometres from one another. Collected samples were stored in 2ml Eppendorf® collection tubes, sealed with Parafilm M® tape and chilled in the field in an effort to mitigate DNA degradation. Once samples were returned to the laboratory, they were stored at -20° C until further processing.

Table 2.1: A summary of the scenarios and sites at which soil and dung samples were collected at the Krommelboog Farm, Beaufort West following the partial integration of the farm's wildlife and livestock populations.

Province	Location	Scenario	Sample Type	Field Name	Lab Code
Western Cape	Beaufort West	Livestock	Dung	PK 23	J.82
Western Cape	Beaufort West	Contact	Dung	DE HOOP SALM 1	J.83
Western Cape	Beaufort West	Wildlife	Dung	DH 21	J.86
Western Cape	Beaufort West	Wildlife	Soil	DH 22	J.87
Western Cape	Beaufort West	Wildlife	Dung	DH 23	J.88
Western Cape	Beaufort West	Livestock	Dung	NOOIT HUIS 2	J.89
Western Cape	Beaufort West	Livestock	Dung	NOOIT HUIS 1	J.90
Western Cape	Beaufort West	Contact	Dung	SALMAN	J.91
Western Cape	Beaufort West	Wildlife	Dung	SALMAN DAM	J.93
Western Cape	Beaufort West	Livestock	Soil	WP 41	J.95
Western Cape	Beaufort West	Livestock	Soil	WP 43	J.96
Western Cape	Beaufort West	Livestock	Soil	HOUSE WP 1.1	J.98

2.2.2 DNA extraction

Soil and dung samples collected at the Krommelboog Farm, were thawed at room temperature. Once thawed, genomic DNA was extracted in triplicate from each of our samples using the commercially available Machery-Nagal Soil DNA kit, according to the instructions provided by the manufacturer (MACHERY-NAGAL GmbH & Co. KG). Extracted bacterial DNA was then stored in 2ml Eppendorf® collection tubes at -20° C until further processing.

2.2.3 V3-V4 amplification, clean up and library preparation

V3-V4 amplification, clean up and library prep was performed by The Agricultural Research Council, on their biotechnology platform in Pretoria, South Africa. Amplification of the V3-V4 hyper-variable region was achieved following the recommendations provided by Illumina (Illumina, 2013). The primers employed for amplification included the 16S forward primer (5'TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG) and the 16S reverse primer (5'GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C), with overhang adaptors TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG (forward) and GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G (reverse) attached. Amplicon PCR was performed in triplicate for all bacterial DNA extracted from each of our samples, the products of which were combined prior to PCR clean up. PCR clean up employed the use of Agencourt AMPure XP Beads (Beckman Coulter Genomics, California, USA) that effectively purified the V3-V4 amplicon from the free primers and primer dimer species and was achieved following recommendations provided by the manufacturer.

Amplicon PCR clean-up was followed by index PCR, which saw the attachment of dual indices and the aforementioned overhang adaptors using the Nextera XT Index Kit. Constructed libraries were then purified using Agencourt AMPure XP beads (Beckman Coulter Genomics, California, USA) prior to quantification with the Qubit fluorometer (Qubit 3.0, Thermo Fisher Scientific, USA).

Following index PCR and clean up, libraries were denatured and samples loaded for MiSeq sequencing. This process included the dilution of purified libraries with re-suspension buffer, effectively normalizing each of the libraries to 4nM. In preparation for the generation of clusters and sequencing, libraries for each of the twelve samples were combined and treated with NaOH and left

to incubate at room temperature for five minutes, thereby denaturing DNA into single strands. DNA was then diluted with hybridization buffer to a final concentration of 2pM. The 10nM PhiX control was then denatured and diluted such that its loading concentration matched that of the amplicon library. The two were then combined, ensuring that the final library mixture contained a minimum of 5.0% PhiX control. The final library was then denatured at 96° prior to loading the library into the MiSeq V3 reagent cartridge (2 X 300bp). Initial analysis and de-multiplexing of reads was performed using the MiSeq reporter software (Illumina, San Diego, CA, USA).

2.2.4 Metagenomic data importation and mapping file generation

The 16S rRNA amplicon sequences obtained from Illumina were analysed using Quantitative Insights into Microbial Ecology (QIIME) version 1.9.1 (Caporaso et al., 2010). Raw reads provided in the FASTQ format were retrieved from the MiSeq Illumina sequencer FTP repository and stored within the appropriate QIIME directory. As is a requirement for most QIIME analyses, a user generated metadata mapping was required such that sample identifiers may be linked to their respective metadata and accessed by the aforementioned the software application. For the purpose of this study, the following mapping file categories were used: SampleID (denoting the sequence headers used in the FASTQ files), BarcodeSequence (denoting the forward and reverse amplicon sequences for each sample respectively), LinkerPrimerSequence (denoting the primer sequences employed for amplification), SampleType (denoting the sample medium i.e. soil or dung), Location (denoting the locale at which each respective samples had been collected) and Description (denoting the sub-regions within which the sample had been collected). Prior to the analysis, the aforementioned mapping file was validated, and any formatting errors corrected. The validated mapping file generated for the purpose of this study may be found in Appendix: 1.

2.2.5 Sequence consolidation, quality control and OTU assignment

Once the mapping file has successfully been validated, pair-ended sequences (FASTQ) for each of the 12 samples collected at the Krommelboog farm were assembled using the PANDASeq (pair-ended assembler for Illumina sequences) with a maximum product size of 460bp (Masella et al., 2012). Adjoined sequences were then individually assessed using the Mothur protocol (Schloss et al., 2009) - providing us an array of statistics related to the sequence reads for each of our samples. The average number of reads per sample were documented, and later used to establish the sampling depth of the analysis. Operational Taxonomic Units (OTU's) were then assigned to our sequences in QIIME 1.9.1 (Caporaso et al., 2010). For the purpose of this study, an open reference OTU picking strategy was employed. According to (Caporaso et al., 2010), open reference OTU picking systems are regarded as a compromised choice between closed reference picking strategies (primarily employed for well-studied microbiomes) and de novo picking strategies (primarily employed for novel microbiome investigation), and are often regarded as the most useful picking system when dealing with environments comprising a combination of known and unknown species. Following the designation of the OTU picking strategy, sequences were clustered at a 90.0% similarity threshold and subsequently aligned to the SILVA 132 16S rRNA gene reference database using PYNAST (Caporaso *et al*., 2009); McDonald *et al*., 2012). Generated OTU clusters were then delineated into phylotypes, which were contrasted against taxonomic information contained in the SILVA 16S rRNA gene reference database, and classified at various taxonomic rankings. This was followed by the rarefication of sequences to a depth of 10,000 reads, and the construction of a phylogenetic tree from the multiple sequences aligned in the previous step. The tree constructed by QIIME 1.9.1 (Caporaso et al., 2010) denotes the relationships exhibited between each of the sequences as a measure of the degree of evolution exhibited by sequences from a common ancestor.

The distributions of various taxa across our study area were graphically represented in the form of stacked bar graphs (taxaplots). Datasets from the aforementioned graphical outputs were subsequently imported into Microsoft® Excel, and filtered to omit any taxa exhibiting a total community contribution of less than 1.0%, or those that were partially classified. Taxaplots were reconstructed using these filtered datasets, and allowed for the effective interpretation of bacterial abundance and diversity across our study area. Ranked taxa data, derived from QIIME 1.9.1 (Caporaso et al., 2010) generated taxaplots, were combined in an effort to elucidate the abundance and diversity of bacterial assemblages across all of the scenarios (contact, livestock and wildlife) sampled at the Krommelboog Farm. The consultation of γ-diversity datasets at the level of phylum, family and genus, allowed for the identification of core microbiome taxa across our study. The same approach was employed for the establishment of α-diversity (*viz*. the diversity of taxa between each of our sampled scenarios (contact, livestock and wildlife).

2.2.6 Statistical analyses of inter-scenario bacterial communities

Community richness estimators (Chao1 and Observed OTU's) and diversity estimators (Shannon, PD whole tree) were calculated in Mothur (Schloss et al., 2009) at a rarefied depth of 10,000 reads. All differences in OTU abundance, diversity, species richness and evenness were established using non-parametric, multiple Students t-Tests (performed using QIIME 1.9.1), the outputs of which were adjusted using the Bonferroni correction. The similarities between scenario-based microbiomes were assessed in Primer v7 (Clarke & Gorley, 2015) using non-metric multi-dimensional scaling (NMDS) with Bray-Curtis distances (Bray-Curtis, 1957). NMDS provided graphical representations (dendrograms) denoting the relationship of bacterial community taxa derived from each of our sampled scenarios. In conjunction with the aforementioned, the inter-scenario bacterial composition was evaluated using a permutation-based hypothesis test, namely, a one-way analysis of similarities

(ANOSIM). Further, a SIMPER (similarity percentage analysis) analysis was performed on ranked scenario taxa data, and allowed for the establishment of contributions of core microbiome taxa to intra-scenario bacterial variation using Bray-Curtis similarity distances (Bray-Curtis, 1957), with a 70.0% cut-off for low contributions. All of the aforementioned α-diversity analyses were performed at phylum level.

2.2.7 Pathogen screening

Pathogen screening was achieved through the consultation of site-specific sample reports provided by Illumina, as well as the OTU table generated during our analysis.

2.3 Results

2.3.1 Delineation of bacterial γ-diversity at the Krommelboog Farm (Wildlife, Livestock and Contact areas)

Our efforts at elucidating the bacterial community structure at the Krommelboog Farm resulted in our twelve samples collectively generating a total of 493,845 sequences, and a total of 17,051 OTU clusters. Of the twelve samples collected, the total number of sequences ranged from 82,111 to 51. The sample exhibiting the greatest number of sequences was sample J.89, a dung sample sourced within wildlife areas at the farm, while the sample exhibiting the fewest sequences was sample J.86, which similarly, had been sourced from a dung sample sourced within the wildlife areas at the farm. The mean number of sequences obtained was 41,153.750, with a standard deviation of 22,723.149. The low sequence count returned by sample J.86, may be attributed to amplification failure, which is often perceived as an indicator of poor sample quality. Consequently, the aforementioned sample was excluded from any further analyses. The SILVA 16S rRNA database was incapable of assigning any form of classification to 1.8% of the taxa detected by our analysis, and included 0.9% of the taxa derived from contact areas, 2.4% of the taxa derived from our livestock areas and finally, 2.5% of the taxa derived from our wildlife areas.

2.3.1.1 Core microbiome taxa identified across all sampled scenarios

Core microbiome taxa (phylum-level) identified at the Krommelboog Farm

Our investigation of bacterial γ-diversity resulted in the detection of 38 bacterial phyla across all sampling areas at the Krommelboog Farm (Fig: 2.2). The core Krommelboog Farm bacterial phyla included: Firmicutes (28.2%), Planctomycetes (19.7%), Actinobacteria (12.5%), the candidate phylum

TM7 (9.0%), Bacteroidetes (8.6%), Euryarchaeota (5.5%), Verrucomicrobia (4.9%), Chloroflexi (3.3%), Proteobacteria (2.4%) and Acidobacteria (1.9%) (Fig 2.2). A comprehensive summary of all detected bacterial phyla, and their respective abundances may be found in Appendix: 2.

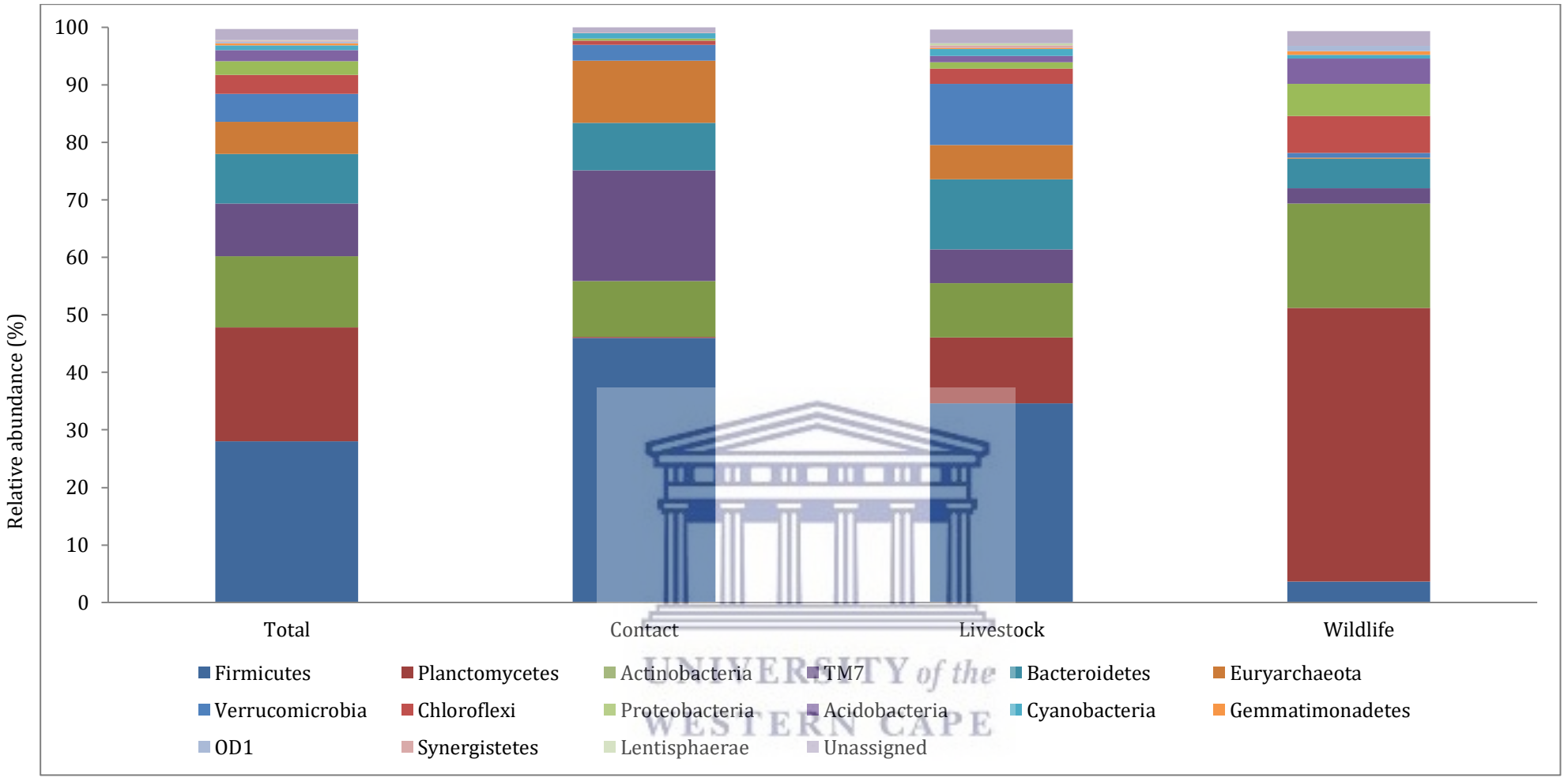


Figure 2.2: Filtered taxaplots (Contact vs. Livestock vs. Wildlife) illustrating the (phylum-level) relative abundance of bacterial taxa detected and classified using the 16S rRNA gene.

Core microbiome taxa (family-level) identified at the Krommelboog Farm

At the rank of family, our phylotypes collectively returned a total of 380 bacterial families (Fig: 2.3), with partially classified taxa accounting for 28.4% of all bacterial families detected within the study area. The core bacterial families included: *Ruminococcaceae* (11.5%), *F16* (8.1%), *Micrococcaceae* (6.8%), *Lachnospiraceae* (5.7%), *Methanobacteriaceae* (5.5%), *Gemmataceae* (4.7%), *Verrucomicrobiaceae* (4.6%), *Coriobacteriaceae* (4.4%), *Bacteroidaceae* (1.9%) and *Ellin6075* (1.6%). A comprehensive summary of all classified bacterial families, and their respective abundances may be found in Appendix: 3.

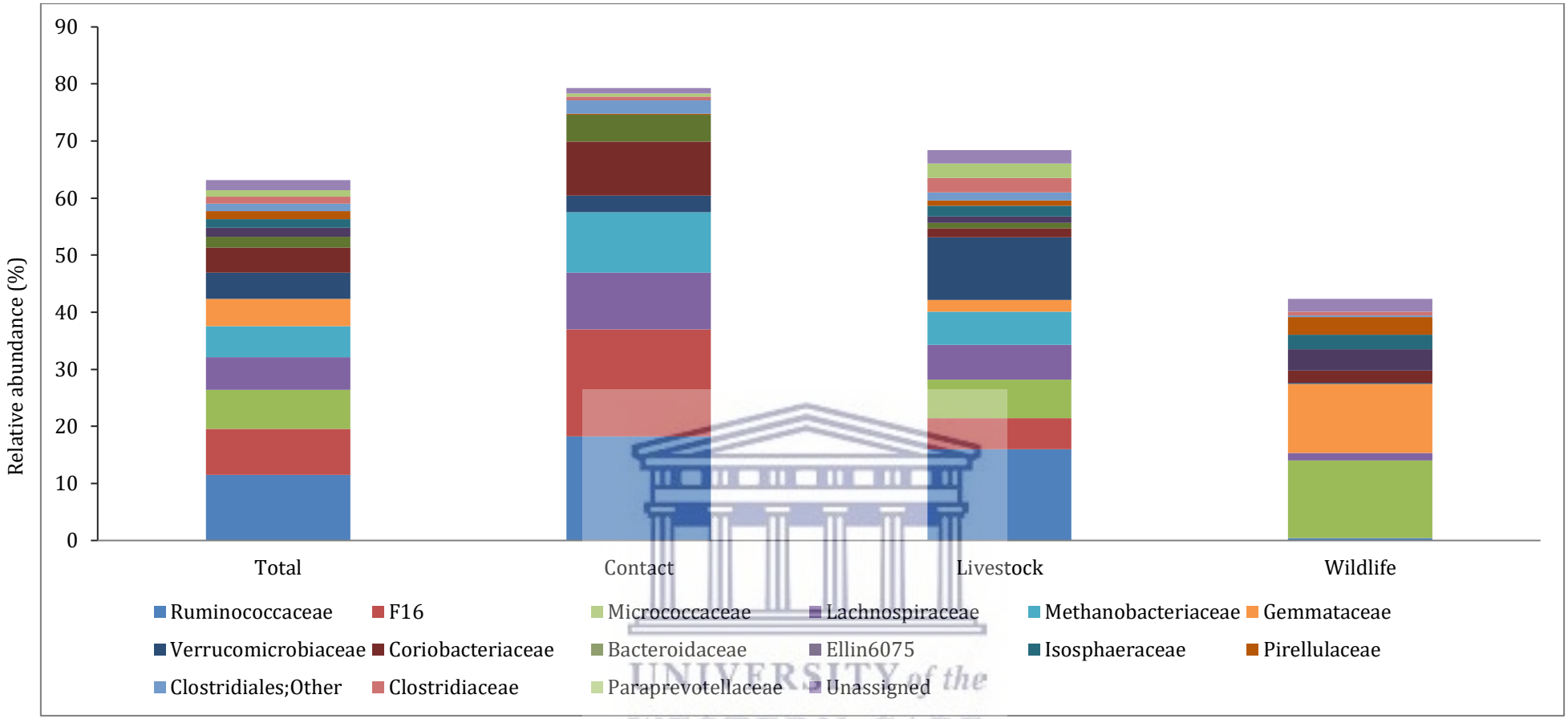


Figure 2.3: Filtered taxaplots illustrating the (family-level) relative abundance of bacterial taxa detected and classified using the 16S rRNA gene. Partially classified families and families with low representation (<0.1%) have been omitted (Total = 29.4; Contact = 17.6%; Livestock = 25.0% &Wildlife = 42.5%).

Core microbiome taxa (genus-level) identified at the Krommelboog Farm

Genus level classification of detected taxa resulted in our phylotypes returning a total of 532 bacterial genera (Fig 2.4), with partially classified taxa accounting for 61.1% of all genera detected within the study area. The core Krommelboog Farm genera included: *Arthrobacter* (6.6%), *Akkermansia* (4.5%), *Gemmata* (4.1%), *Methanosphaera* (3.5%), *Eggerthella* (2.4%), *Methanobrevibacter* (2.0%), *Bacteroides* (1.4%), *Oscillospira* (1.0%), *Clostridium* (0.9%) and *Butyrivibrio* (0.9%). A comprehensive summary of all classified bacterial genera, and their respective abundances may be found in Appendix: 4.

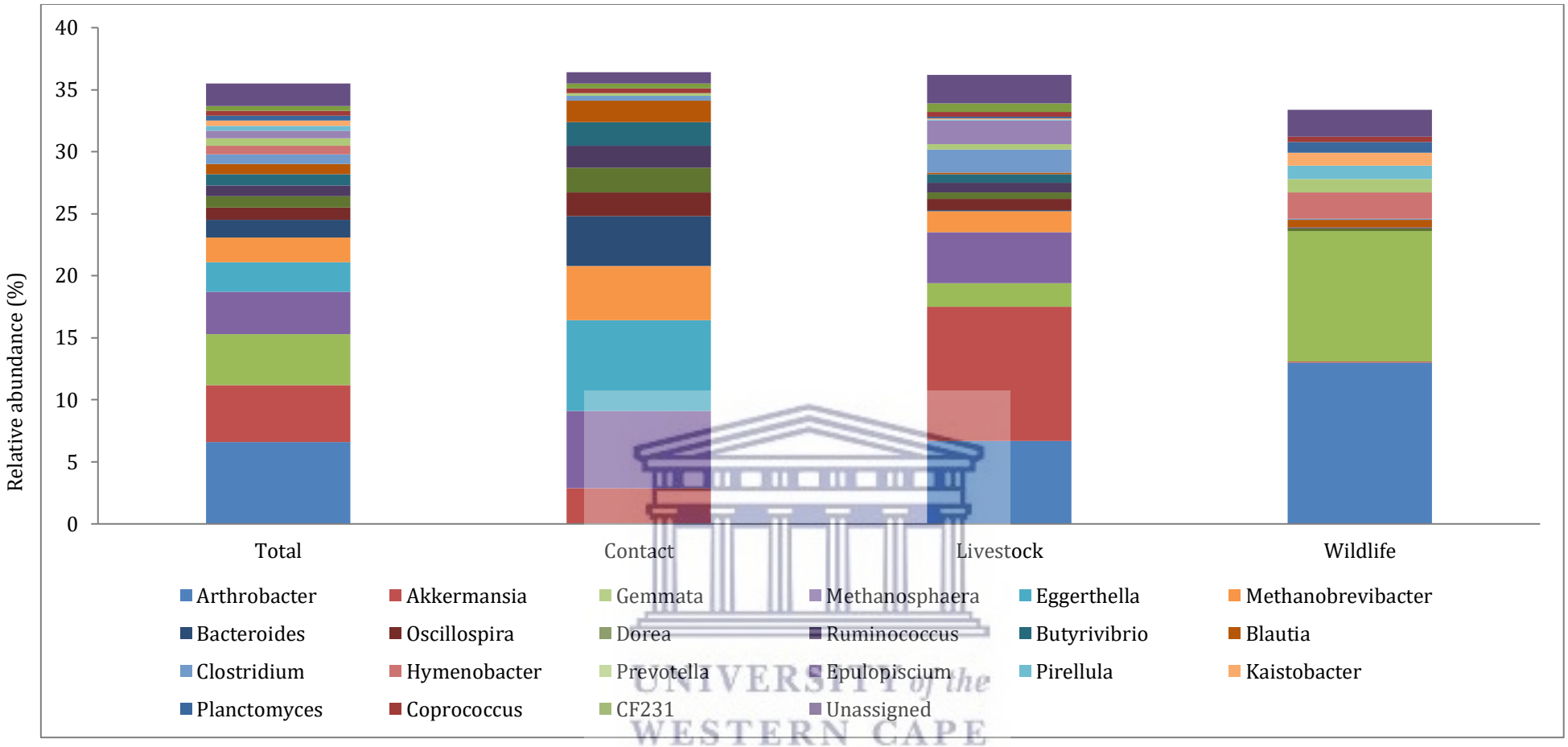


Figure 2.4: Filtered taxaplots illustrating the (genus-level) relative abundance of bacterial taxa detected and classified using the 16S rRNA gene. Partially classified genera and those genera exhibiting low representation (>0.1%) have been omitted (Total = 61.1%; Contact = 61.6%; Livestock = 60.2% & Wildlife = 62.4%).

2.3.2 Delineating *A* and *B*-diversity at the Krommelboog Farm

2.3.2.1 Core microbiome taxa (phylum-level) identified by scenario type

Samples derived from contact scenarios at the Krommelboog farm returned a total of 11 bacterial phyla - the fewest phyla for all of our sampled scenarios. Core contact scenario derived phyla included: Firmicutes (46.0%), TM7 (19.2%), Euryarchaeota (10.8%), Actinobacteria (9.7%), Bacteroidetes (8.3%), Verrucomicrobia (2.8%), Cyanobacteria (0.9%), Chloroflexi (0.7%), Proteobacteria (0.4%), Planctomycetes (0.2%) and Synergistetes (0.1%). Samples derived from our livestock scenarios returned a total of 17 bacterial phyla. Core livestock derived phyla included: Firmicutes (34.6%), Bacteroidetes (12.2%), Planctomycetes (11.5%), Verrucomicrobia (10.7%), Actinobacteria (9.4%), TM7 (5.9%), Euryarchaeota (5.9%), Chloroflexi (2.6%), Cyanobacteria (1.2%) and Proteobacteria (1.1%). Samples collected within wildlife scenarios returned a total of 18 bacterial phyla - the greatest number of phyla across all sampled scenarios. Core wildlife scenario derived phyla included: Planctomycetes (47.6%), Actinobacteria (18.2%), Chloroflexi (6.4%), Proteobacteria (5.6%), Bacteroidetes (5.2%), Acidobacteria (4.4%), Firmicutes (3.6%), TM7 (2.6%), Verrucomicrobia (0.9%) and OD1 (0.9%).

2.3.2.2 Core microbiome taxa (family-level) identified by scenario type

Samples derived from contact scenarios within our study area returned a total of 28 bacterial families; the fewest number of families across all of our sampled scenarios. Core contact scenario families included: *F16* (19.2%), *Ruminococcaceae* (18.0%), *Methanobacteriaceae* (10.8%), *Lachnospiraceae* (9.9%), *Coriobacteriaceae* (9.4%),

Bacteroidaceae (4.7%), *Verrucomicrobiaceae* (2.8%), *S24-7* (1.5%), *Clostridiaceae* (0.7%) and *Anaerolineaceae* (0.7%) (Fig 2.3). Samples sourced and sequenced from livestock scenarios at the farm, returned a total of 51 families. Core taxa detected and classified within our livestock areas included: *Ruminococcaceae* (15.9%), *Verrucomicrobiaceae* (10.6%), *Micrococcaceae* (6.8%), *Lachnospiraceae* (6.0%), *Methanobacteriaceae* (5.9%), *F16* (5.6%), *Clostridiaceae* (2.6%), *Paraprevotellaceae* (2.6%), *Gemmataceae* (2.1%) and *Isophaeraceae* (1.8%). (Fig 2.3). Within our wildlife areas, samples returned a total of 57 bacterial families – the greatest number of detected families across all sampled scenarios. Samples derived from this scenario included the following core families: *Micrococcaceae* (13.6%), *Gemmataceae* (12.1%), *Ellin6075* (3.7%), *Pirellulaceae* (3.1%), *Isophaeraceae* (2.6%), *Cytophagaceae* (2.6%), *Sphingomonadaceae* (2.4%), *Coriobacteriaceae* (2.2%), *Lachnospiraceae* (1.3%) and *Caldilineaceae* (1.3%). (Fig 2.3). It should be noted that partially classified taxa accounted for 17.6% (contact), 42.5% (wildlife) and 25.0% (livestock) of all families detected across our study area.

2.3.2.3 Core microbiome taxa (genus-level) identified by scenario type

Our investigation of genera-level bacterial diversity across our sampled scenarios returned the following information: Contact areas returned a total of 21 bacterial genera – the fewest bacterial genera across all sampled scenarios. Core genera identified within this scenario included: *Eggerthella* (7.3%), *Methanosphaera* (6.2%), *Methanobrevibacter* (4.4%), *Bacteroides* (4.0%), *Akkermansia* (2.9%), *Dorea* (2.0%), *Oscillospira* (1.9%), *Butyrivibrio* (1.9%), *Ruminococcus* (1.8%) and *Blautia* (1.7%) (Fig 2.4).

Samples sourced from wildlife areas returned a total of 46 bacterial genera – the greatest number of genera across all of our sampled scenarios. Core bacterial genera within this scenario included: *Arthrobacter* (13.0%), *Gemmata* (10.5%), *Hymenobacter* (2.1%), *Prevotella* (1.1%), *Pirellula* (1.1%), *Collinsella* (1.0%), *Kaistobacter* (1.0%), *Planctomyces* (0.9%), *Blautia* (0.6%) and *Anaerolinea* (0.6%) (Fig 2.4). Finally, samples collected within livestock areas at the farm returned a total of 37 bacterial genera, and comprised the following core families: *Akkermansia* (10.8%), *Arthrobacter* (6.7%), *Methanosphaera* (4.1%), *Clostridium* (1.9%), *Gemmata* (1.9%), *Epulopiscium* (1.9%), *Methanobrevibacter* (1.7%), *Oscillospira* (0.9%), *Ruminococcus* (0.8%) and *Butyrivibrio* (0.7%) (Fig 2.4). Partially classified taxa accounted for 61.6% (contact), 62.4% (wildlife) and 60.2% (livestock) of all bacterial genera detected across all scenarios.

2.3.3.1 *A*-diversity rarefaction plots: Species diversity and richness metrics

A-diversity rarefaction plots for our four metrics (Chao1, Shannon, PD whole tree, Observed OTU's) (Fig 2.5), indicated a sufficient sampling effort, as curves tended to stabilize – an indication that the sequences detected offer an adequate representation of the bacterial identity for our sampled scenarios.

In terms of species richness, our indexes (Chao/Observed OTU'S) revealed the following information: The majority of the unique OTU's detected across our sampling scenarios, were derived from areas that were exclusively populated by wildlife factions. Our results show high levels of similarity and overlap between wildlife, livestock and contact area derived samples in terms of species richness (Chao1), and bacterial diversity (Shannon, PD whole tree).

The aforementioned sample similarity and overlap between sampled scenarios was further corroborated by our pairwise comparison of sampled scenarios using non-parametric multiple Student's t-Tests (999 Monte Carlo permutations), which indicated no significant differences between bacterial communities (Table: 2.2).

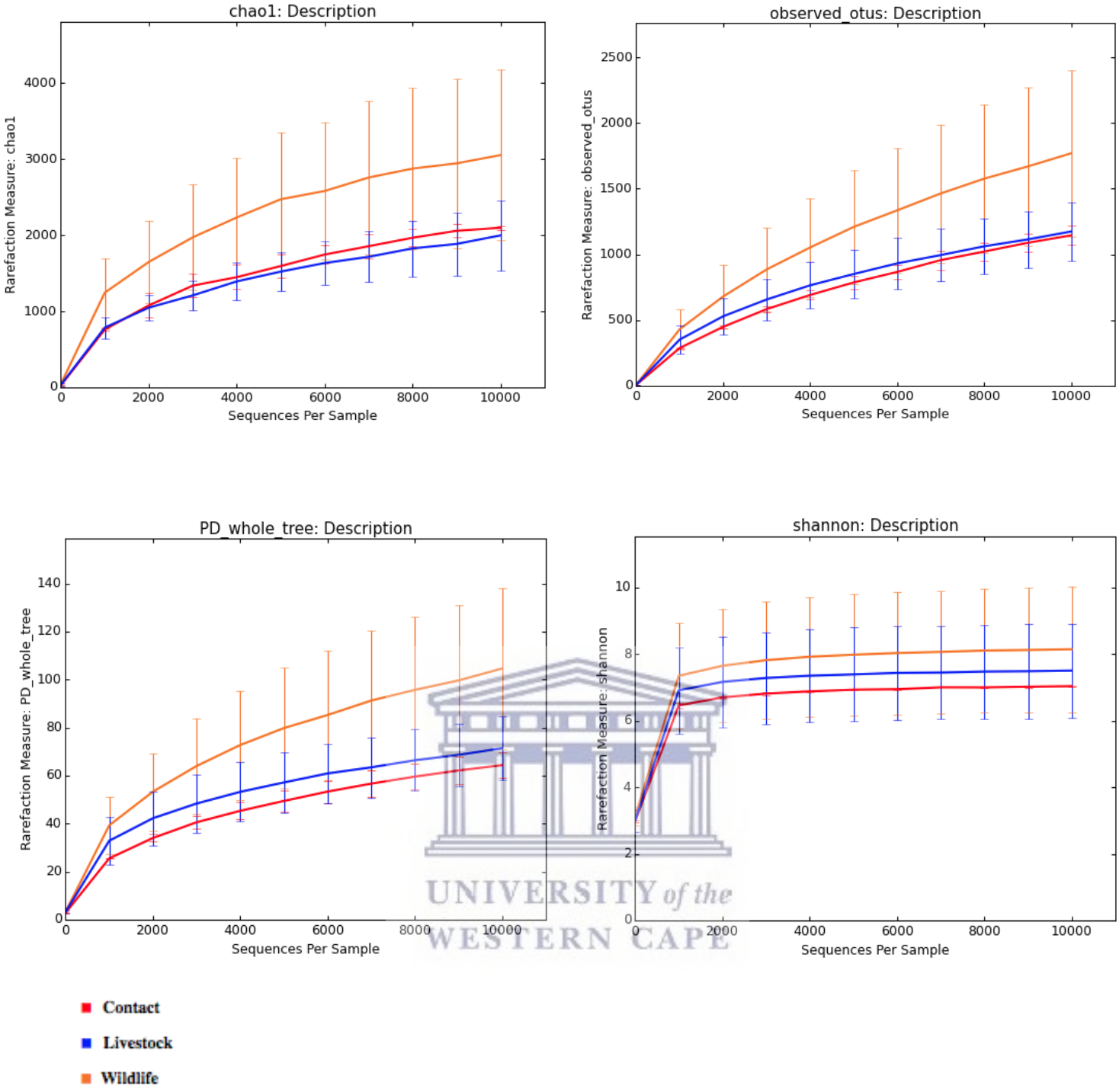


Figure 2.5: Rarefaction plots denoting differences in α-diversity between sampled scenarios at the Krommelboog Farm. The Chao1 metric (top left) estimates diversity as a measure of species abundance, Observed OTU's (top right), expresses species richness as a function of the number of operational taxonomic units (OTU's) detected across samples, PD whole tree (bottom left) estimates diversity as a function of the sum of all phylogenetic distances between detected between taxa while the Shannon metric (bottom right) estimates diversity as a function of entropy.

Table 2.2: Scenario-based pairwise comparisons of species richness (Chao1), OTU abundance (Observed OTU's), phylogenetic diversity (PD whole tree) and diversity (Shannon) using non-parametric multiple Student's t-Tests (999 Monte Carlo permutations), with Bonferroni adjusted outputs (performed using QIIME 1.9.1).

Chao1

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t statistic	p-value
Wildlife	Livestock	2997.52	1117.94	1933.53	473.20	1.76	0.32
Wildlife	Contact	2997.52	1117.94	2035.25	157.61	0.93	0.98
Contact	Livestock	2035.254	157.61	1933.53	473.20	0.25	1.0

Observed OTU's

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Wildlife	Livestock	1758.13	612.83	1159.01	243.81	1.84	0.36
Wildlife	Contact	1758.13	612.83	1134.00	135.00	1.09	1.00
Contact	Livestock	1134.00	135.00	1159.01	243.81	0.11	1.00

PD whole tree

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Wildlife	Livestock	102.66	32.88	69.92	13.95	1.84	0.33
Wildlife	Contact	102.66	32.88	63.24	6.65	1.29	1.00
Contact	Livestock	63.24	6.65	69.92	13.95	0.56	1.00

Shannon

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Wildlife	Livestock	8.10	1.84	7.41	1.51	0.52	1.00
Wildlife	Contact	8.10	1.84	6.95	0.11	0.68	1.00
Contact	Livestock	6.95	0.11	7.41	1.51	0.36	1.00

2.3.3.2 *A* and *B*-diversity: ANOSIM, NMDS and SIMPER

A-diversity: ANOSIM & NMDS

Rarefied OTU data from each of our sampled scenarios were investigated *in silico*, using a one-way analysis of similarities (ANOSIM) employing the use of the Bray-Curtis similarity index. Analysis of phylum-level OTU's derived from each of the three sampled scenarios (contact, livestock & wildlife) indicated high similarity in terms of OTU composition ($p = 0.28$, $R = 0.064$). This finding was further corroborated by NMDS (using the Bray-Curtis similarity index), which delineated the relationships of sampled areas and their respective samples, as a function of their OTU composition (Fig 2.6). In addition to the aforementioned, our SIMPER analysis (one-way) quantified the dissimilarities between our sampled scenarios as follows: Livestock and contact areas exhibited an average OTU composition dissimilarity of 51.44%, a result of the contributions of the following core phyla: Firmicutes (23.96%), Planctomycetes (15.55%), Actinobacteria (14.53%), TM7 (13.71%) and Bacteroidetes (8.72%). Livestock and wildlife areas exhibited an average OTU composition dissimilarity of 69.48%, and was determined by: Planctomycetes (27.02%), Firmicutes (50.56%), Actinobacteria (17.87%) and Bacteroidetes (6.21%). Finally, a comparison between contact and wildlife areas revealed an average dissimilarity of 80.93%, which was largely attributed to the contributions of Planctomycetes (26.88%), Firmicutes (52.83%), Actinobacteria (14.95%), and TM7 (9.95%).

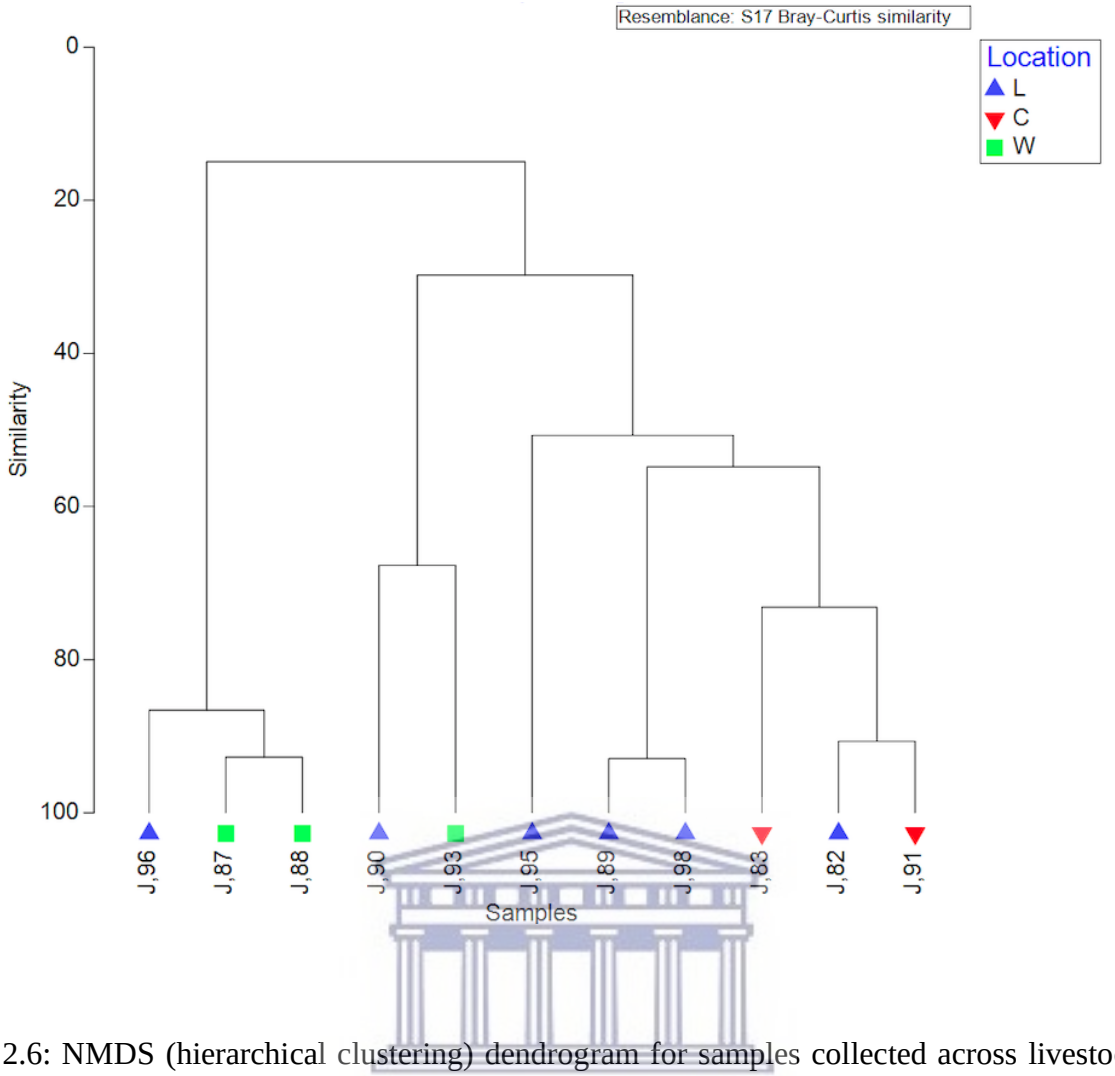


Figure 2.6: NMDS (hierarchical clustering) dendrogram for samples collected across livestock (L), contact (C) and wildlife (W) scenarios at the Krommelboog farm, in which samples were grouped according to OTU composition, using the Bray-Curtis similarity index.

B-diversity: OTU Composition

In terms of β diversity, our SIMPER analysis yielded the following results: The greatest level of intra-scenario bacterial OTU composition similarity was observed within "contact" areas, where the average OTU similarity was 71.99%. The similarity demonstrated among these samples was determined by the contributions of Firmicutes (61.52%) and TM7 (18.40%). This was followed by the "wildlife" areas which demonstrated an average OTU similarity of 38.52%, and determined by the contributions of Planctomycetes (58.38%), Proteobacteria (10.22%) and Chloroflexi (7.58%). Finally, the

"livestock" areas at the Krommelboog farm demonstrated the lowest similarity between samples, with an average OTU similarity of 35.23%, and was largely determined by the contributions of Firmicutes (49.46%), Planctomycetes (13.58%) and Bacteroidetes (7.67%).

2.3.4 Detected pathogenic bacteria

A total of five pathogenic strains of bacteria were detected across the scenarios sampled at the Krommelboog Farm, Beaufort West (Table 2.3). Pathogenic bacteria were identified using sample summary reports provided by Illumina, each of which provided us with a breakdown of the most prevalent bacteria for each site within our three sampled scenarios. The five identified pathogenic strains of bacteria, namely, *Clostridium gasigenes*, *Exiguobacterium sibiricum*, *Escherichia albertii*, *Egerthella lenta* and *Lactobacillus acidophilus*, constituted 2.2% of all the fully classified bacteria genera detected. The greatest number of pathogens were detected within a single dung sample (J.83) collected within the contact area at the Krommelboog Farm, and included the taxa: *Escherichia albertii*, *Egerthella lenta* and *Lactobacillus acidophilus*. The livestock scenario at the farm, a single pathogen was detected, namely, *Clostridium gasigenes*, which was isolated from a soil sample (J.95). Similarly, our investigation of the wildlife scenario at the farm, led to the detection of a single pathogen *Exiguobacterium sibiricum*, which was isolated from a dung sample (J.93). With that being said, contact scenarios at the Krommelboog Farm returned an average of 1.5 pathogens per sample (N=2), wildlife areas returned an average of 0.33 pathogens per sample (N=3) and livestock returned an average of 0.166 pathogens per sample.

Table 2.3: Summary of pathogenic bacteria detected within soil and faecal samples within contact, livestock and wildlife scenarios at the Krommelboog Farm, Beaufort West.

Species	Scenario	Sample Type	Field Name	Lab Code
Escherichia albertii	Contact	Dung	DE HOOP SALM 1	J.83
Egerthella lenta	Contact	Dung	DE HOOP SALM 1	J.83
Lactobacillus acidophilus	Contact	Dung	DE HOOP SALM 1	J.83
Exiguobacterium sibiricum	Wildlife	Dung	SALMAN DAM	J.93
Clostridium gasigenes	Livestock	Soil	WP 41	J.95

2.4 Discussion

Our analysis of soil and dung derived sequences provided us with a detailed overview of the bacterial communities present at various scenarios sampled ta the Krommelboog Farm. The findings of this paper were generated through the application of NGS technology targeting the 16S rRNA gene, in conjunction with appropriate *in silico* analyses of sequences using QIIME 1.9.1 (Caporaso et al., 2010)

Our investigations into the bacterial community at the Krommelboog Farm resulted in the detection of 38 bacterial phyla, comprising a total of 380 families and 532 genera. Our investigations of wildlife, livestock and contact scenarios at the farm identified the Firmicutes (28.0%) and Planctomycetes (19.8%) as the dominant phyla - collectively accounting for 47.8% of bacterial phyla detected in this study (Fig 2.2). Observed patterns observed within detected bacterial phyla were subsequently carried over to lower levels of classification. Despite the detection of 380 bacterial families, our samples were dominated by the presence of five families, namely, *Rumminococcaceae*, *Micrococcaceae*, *Lachnospiraceae*, *Methanobacteriaceae* and *Gemmataceae*, which collectively represented a mere 1.3% of all detected families, but accounted for 42.3% of the family-level OTU's returned by our analysis (Fig 2.3). Similarly, genus-level bacterial taxaplots were dominated by five bacterial genera,

that represented 0.94% of all detected genera, but accounted for 21.1% of all genus-level OTU's detected by our investigation, and included the genera: *Arthrobacter*, *Akkermansia*, *Gemmata*, *Methanosphaera* and *Eggerthella* (Fig 2.4).

2.4.1 Krommelboog bacterial community composition: *A*-diversity & *B*-diversity

Our investigations into the α-diversity of bacterial communities at the Krommelboog Farm provided us with invaluable insights pertaining to the abundance, distribution and diversity of bacterial assemblages between sampled scenarios. *A*-diversity (Fig 2.5) rarefaction plots, indicate an adequate sampling effort, which is demonstrated by the stabilization of our curves, and is an indication that sequences generated by our investigation offer an accurate representation of the bacterial identities of sampled scenarios. Statistical analyses of bacterial community OTU's indicated no significant differences in bacterial diversity (PD whole tree; Shannon Index) or species richness (Observed OTU's; Chao1) between our sampled scenarios ($P > 0.05$) (Table 2.2). These outputs were corroborated by our ANOSIM, which showed high levels of OTU homogeneity between our "contact", "wildlife" and "livestock" scenarios, with high levels of OTU overlap ($p = 0.28$, $R = 0.064$). Further support was drawn from our NMDS (hierarchical clustering) dendrograms, which showed high levels of OTU similarity between scenarios (Fig 2.6). It should however be noted that the statistical power of our non-parametric pairwise analyses may potentially have been diminished by the low number of samples collected within contact scenarios (N=2) and wildlife scenarios (N=3). Despite the absence of significant differences in bacterial diversity between our sampled areas, phylum-level investigations revealed notable differences in the composition of bacterial communities between each of our sampled scenarios. Our analysis of scenario specific (β-diversity) community composition, indicated that our "contact" areas demonstrated high levels of OTU similarity (71.99%), followed by "wildlife" areas (38.52%), and finally our livestock areas (35.23%).

Our α-diversity analyses indicate that our sampled scenarios demonstrated a tendency to be dominated by two bacterial phyla in particular, namely, Planctomycetes and Firmicutes, which is well evidenced by taxaplots depicting the relative abundance of bacterial assemblages across each of the farm's scenarios (Fig 2.2). Furthermore, Planctomycetes and its members demonstrated greater contributions to soil bacterial communities within "wildlife" areas at the Krommelboog Farm, contributing 47.6% to the scenario's total bacterial diversity. Contact scenarios at the Krommelboog Farm, demonstrated the lowest levels of Planctomycetes abundance, where members of the phyla contributed a mere 0.2% to the identity of the scenario's bacterial microbiome. In contrast, Firmicutes members had the highest representation within the farm's contact areas, where its members contributed 46.0% to the identity of the scenario's bacterial identity. This was followed by samples derived from the farm's livestock areas, where Firmicutes members contributed 34.6% to the area's bacterial community composition. It should be noted that wildlife areas exhibited the lowest abundance of Firmicutes members, where its members contributed a mere 3.6% to the scenario's bacterial community. In order to better understand the interaction between members of the aforementioned phyla, it is imperative to gain an understanding of the underlying physiology of these taxa and their interactions with their physical environments.

Planctomycetes: Physiology, features and their interaction with the environment

The phylum Planctomycetes was first proposed in 2001 (Garrity & Holt, 2001). The taxonomic status of the group underwent revision in 2006, when it was designated to a superphylum that integrated the phyla Planctomycetes, Chlamydiae and Verrucomicrobia (Superphylum PVC, Wagener & Horn, 2006). Under its current classification, Planctomycetes comprises three orders: Physcisphaerales, Planctomycetes and "Candidatus Brocadiales", and include a total of 11 described genera and 14 species. Despite their ubiquity within terrestrial (e.g., tundra, forest, cultivated pastures and thermal

soils) and marine environments across the globe, the ecological significance of this taxa remains poorly understood (Buckley et al., 2006). What is known about the phylum and its members is that they exhibit an array of features that sets them apart from other *Bacteria* (Ward et al., 2006). The most notable of these features include: the absence of peptidoglycan from their cell walls (Ward et al., 2006), compartmentalized internal cellular structure and an ability to divide through budding (Fuerst, 1995; Lindsay, Webb & Fuerst, 1997; Lindsay et al., 2001). Furthermore, this group of bacteria is known for their slow growth rates (Fuerst, 1995), which is further suppressed in the presence of fast-growing bacterial forms (Lage & Bondoso, 2012). Moreover, members of the phyla are reported to exhibit higher levels of diversity in moist anoxic bulk soils (Derakshani, Lukow & Liesack, 2001).

A study conducted by Buckley & Schmidt, (2003), investigated the dynamics and diversity of bacterial communities within agricultural ecosystems, the outputs of which, indicated that abundance of Planctomycetes populations within soils, may be correlated with land use regimes. Further investigations in which Planctomycetes diversity was measured against soil history (i.e., soil disturbance), indicated that undisturbed soils tend to exhibit greater abundances of Planctomycetes when contrasted against soils that had been impacted by disturbance (Buckley et al., 2006). The physical causes of induced soil disturbance are well documented, even in the most well managed grazing systems (Hiltbrunner et al., 2012). The major cause of soil disturbance within animal production systems includes trampling, wallowing and pawing by animals, each of which have the capacity to disturb soils crusts to a depth of 10 cm (Fleischner, 1994; Belsky & Gelbard, 2000).

Based on these findings, we postulate that the abundance of Planctomycetes within our samples may serve as an accurate indicator of soil disturbance within sampled areas. Phylum level taxaplots (Fig 2.3) indicate suppressed levels of Planctomycetes within “contact” and “livestock” samples, while “wildlife’ samples were dominated by this phylum and its members.

In order to better understand the dynamics of animal-induced soil disturbance, we refer to Dubeux & Sollenberger, 2020, in which the stocking rate of agricultural animals were shown to alter soils by altering their physico-chemical properties. A closer look at the stocking rates within our sampled scenarios at the Krommelboog Farm, provided us further insight on this matter. Aerial surveys conducted by the Landmark Foundation, indicate that farms livestock population significantly outnumber its wildlife populations (1,600 head of livestock to 380 head of wildlife). While the sizeable livestock population at the Krommelboog Farm is afforded a greater area of occupation overall, these animals typically occupy smaller areas when housed within shepherd camps, thereby elevating both the stocking rate and intensity of animal-induced soil disturbance within these areas. In contrast, the limited wildlife population at the farm, is afforded a smaller area for occupation (see Fig 2.1), but enjoys a greater area of available for occupation. The aforementioned arrangement means that "wildlife" areas tend to demonstrate lower stocking rates, and equally lower levels of animal induced soil disturbance. "Contact" scenario samples demonstrated the highest levels of Planctomycetes suppression, which may suggest high levels of animal-induced soil disturbance following the integration of wild and domestic populations within this area.

Firmicutes: Physiology, features and their interaction with the environment

The most prevalent group of bacteria across our sampled scenarios was the bacterial phylum Firmicutes. According to studies by Hugenholtz, (2002), the Firmicutes are the second most abundant bacterial, and comprises three classes: *Bacilli, Clostridia* and *Erysipelotrichi* (Logan & de Vos, 2009). The *Bacilli* and *Clostridia* are regarded as a paraphyletic grouping within Firmicutes, and are characterized by their endospore forming capabilities, which sets them apart from the *Erysipelotrichi* (Logan & De Vos, 2009). According to studies by Nicholson et al., (2002) and Yung (2006), the sporulation ability of the aforementioned classes offers its members an enhanced survival advantage,

and together with their broad metabolic diversity, may account for their ubiquitous presence across a variety of environmental conditions.

Observed patterns in the distribution and abundance of the Firmicutes across our sampled scenarios indicate a tendency for Firmicutes to occupy areas at the farm that are associated with livestock populations and areas in which animals have been integrated (Fig 2.2). The dominance demonstrated by Firmicutes and its members within samples sourced from scenarios at the Krommelboog Farm, could in part, be attributed to their underlying physiology (i.e., spore forming capabilities and gram-positive walls) of EFF Firmicutes (i.e., *Clostridia* and Bacilli). The sporulation capabilities of the EFF allow these forms to persist within environments where other bacterial forms have otherwise been suppressed by desiccation stresses and other disruptions to the physical environment (Onyenwoke et al., 2004; Schimel, Balser & Wallenstein, 2007). These findings offer insights into the impact of animal stocking rates and animal-induced disturbances on bacterial community composition, which is well evidenced by the displacement of Planctomycetes and its members, by resilient forms of endospore-forming Firmicutes, within the "contact" and "livestock" areas at the farm.

2.4.2 Pathogen screening at the Krommelboog Farm

The summary reports issued by Illumina, and the user-generated OTU table served as the basis for pathogenic bacteria detection in this study, with the former including the seven most prevalent bacterial species detected at each sampling site. Of the 77 most prevalent bacteria detected across our study area, 6.5% proved to be pathogenic, with the majority thereof being detected within contact areas at the Krommelboog Farm. Our screening effort at the Krommelboog Farm support the postulations of Bester & Penzhorn, 2002, who have emphasized the risk of wild and domestic animal integration. Despite the clinical implications associated with pathogens (Table: 2.4), the low contributions of these

taxa to the total number of OTU's detected, suggests that they are unlikely to incur health and economic losses at the Krommelboog Farm.

Table 2.4: Summary of pathogenic bacteria detected within soil and faecal samples within contact, livestock and wildlife scenarios at the Krommelboog Farm, Beaufort West, and their clinical implications.

Species	Scenario	Clinical Implications
Escherichia albertii	Contact	Gastro-enteritis (Ooka et al., 2013)
Egerthella lenta	Contact	Blood stream infection (Venugopal et al., 2012; Bo et al., 2020)
Lactobacillus acidophilus	Contact	Opportunistic pathogen, sepsis (Thompson et al., 2001)
Exiguobacterium sibiricum	Wildlife	Cutaneous lesions (Tena et al., 2014)
Clostridium gasigenes	Livestock	Vacuum-packed meat spoilage, food poisoning (Esteves et al., 2022)

2.5 Conclusion

Our analysis of soil and dung derived samples from wildlife, livestock and contact scenarios at the Krommelboog Farm, offered us the opportunity to delineate microbial community structure, while simultaneously offering us the opportunity to screen our samples pathogens capable of incurring health and economic losses at our study site.

While our statistical analysis of the data showed no significant differences in in bacterial diversity and abundance between our sampled scenarios, the findings of this study highlight the propensity for bacterial community compositions to be offset by animal-induced soil disturbance. Patterns in bacterial abundance and distribution between our sampled scenarios indicate a tendency for sensitive bacteria (e.g., Planctomycetes), to suffer declines in response to increased soil disturbances. These soil disturbances come as a consequence of high stocking rates within selected scenarios at The Krommelboog farm, which has the ability to affect the physico-chemical properties of soils that govern bacterial diversity. The observed declines in these populations have facilitated the proliferation of other bacterial suites (i.e., Firmicutes) which possess physiological adaptations that allow its members to persist and overcome unfavourable conditions and disturbance. While this may lead one to postulate that animal integration may invariably led to declines in soil bacterial diversity - consequence of animal-induced soil disturbance - the stocking rate of animals appears to be the factor dictating soil bacterial diversity. Our efforts at screening the environment for potential threats, lead to the discovery of five pathogen strains of bacteria, namely, *Exiguobacterium sibiricum*, *Clostridium gasigenes*, *Escherichia albertii*, *Lactobacillus acidophilus* and *Egerthella lenta*, the majority of which were detected within "contact" areas. While these pathogens do carry a threat of pathogenesis, they are unlikely to pose a threat to human/animal health and economic activities at the Krommelboog Farm. Nevertheless, the outcomes of this study have emphasized the importance of routine zoonotic

surveillance at the wildlife/livestock interface, where incidents of disease transmission may be elevated.

In conclusion, the obvious economic advantages and practicality associated with mixed animal farming means that this practice will continue to draw the support of South African farmers. While this has generated useful outputs that offer us a better understanding of mixed animal farming, and the impact that it has on both disease prevalence and soil bacterial diversity. Further studies are needed to gain a better grasp of the risks and benefits associated with expanding traditional livestock systems to include wildlife.

Chapter 3

Evaluating the impact of the long-term integration of wildlife and livestock on the prevalence of pathogens and bacterial community composition of soils at the Krommelboog Farm, using high-throughput 16S rRNA sequencing

3.1 Introduction

Over the past 30 years, the world has witnessed an increased incidence in cases associated with the transmission of novel and known zoonotic diseases (Wilcox & Gubler, 2005). By definition, zoonotic diseases are described as infectious diseases, capable of moving in and between wild or domesticated animals and human beings (Slingenbergh et al., 2004). According to studies by Lloyd-Smith et al., (2009), zoonotic disease may be categorized on the basis of their route of transmission (e.g., foodborne or vector-borne), the degree of transmissibility between human beings and the pathogen type. Zoonoses may be bacterial, parasitic, viral, or in certain instances involve a variety of uncommon organisms such as protozoa and fungi (Haydon et al., 2002), with the likelihood of a zoonotic pathogen being associated with either emerging or re-emerging infectious diseases being the highest among viruses and the lowest among helminths (worm-like parasite) (Woolhouse & Gowtage-Sequeria, 2005). Generally speaking, the transmission of zoonotic diseases from animals to humans tends to occur as human beings are integrated into "disease-ecological cycles" where they serve as accidental or "spill-over hosts" for pathogens that are generally contained within animal or insect reservoirs (Kayali et al., 2003; Schelling et al., 2003).

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In order to improve our understanding of zoonoses, and its contributions to declines in human health, we refer to Jones et al., (2008), in which it was reported that zoonotic pathogens constitute 61.0% of all human pathogens, and that 72.0% of emerging and re-emerging infectious diseases are in fact also

zoonotic in nature. The annual burden imposed by zoonotic diseases is estimated to be in excess of 2.5 billion cases, resulting in the deaths of at least 2.7 million individuals (Grace et al., 2012). Moreover, zoonotic diseases have demonstrated an ability to disproportionately affect developing or impoverished countries, where the frequency and intensity of outbreaks exceed those observed within developed countries (King, 2011; Grace et al., 2017).

While a significant amount of attention has been devoted towards the study of animal borne diseases throughout the past half-century, our ability to mitigate the impacts of zoonoses remains limited by our poor understanding of disease dynamics at the wildlife/livestock interface (Rhyan & Spracker, 2010). Leading up to and following the turn of the century, the world has witnessed notable inclines in the proliferation of emerging and re-emerging diseases (Hui, 2006). Throughout this period, it is believed that up to 75.0% of all reported incidents relating to diseases have been linked to wildlife (Woolhouse, 2002). According to studies, recent inclines in wildlife-borne diseases have prompted new questions relating to the epidemiology and pathogenesis and have been accompanied by resolute strides towards the development of effective wildlife-disease management strategies (Vaske, Shelby & Needham, 2009; Rhyan & Spracker, 2010).

Jones et al., (2008) attributes wildlife's involvement in recent disease outbreaks to a multitude of factors - many of which have manifested as a by-product of anthropogenic activities. Many zoonoses have been linked to broad-scale changes in land use, that have demonstrated an ability to alter biodiversity, the interactions between humans, animal hosts, and pathogens, and in certain instances drive epidemiological patterns (Bengis et al., 2004; Patz et al., 2004; Cunningham, 2005; Karesh et al., 2012; Jones et al., 2013). Interestingly enough, many have also attributed the recent inclines in disease emergence to the newfound attraction and interest in wildlife, which has led to the expansion of wildlife-based industries across the globe (Vaske, Shelby & Needham, 2009; Rhyan & Spracker,

2010). These industries require the frequent translocation of wildlife species, and are also responsible for bringing humans into close proximity with potential disease reservoirs (Rhyan & Spracker, 2010).

Previous studies have demonstrated a tendency to overemphasize the epidemiological role of wildlife in the transmission of diseases at the wildlife/livestock interface, where they are frequently regarded as maintenance, spillback/spill over or dead-end hosts (Bengis et al., 2004; Siembieda et al., 2011). According to Chardonnet et al., (2002), the aforementioned stance reflects the anthropogenic bias placed on wildlife, and exemplifies our tendency to overlook the vital roles played by wildlife within various ecosystems. More accurately, disease transmission at the "interface" is dynamic and bi-directional, and allows for the transmission of pathogens between and within populations of both wildlife and livestock (Bengis et al., 2004).

The bi-directional threat of disease transmission is well evidenced in literature. Wild ruminants have been identified as maintenance hosts for a variety of diseases, many of which have the ability to increase disease-related mortalities that compromise livestock production (Bengis, Kock & Fischer, 2002). This may be seen in the interactions between tsetse flies and wildlife (e.g., buffalo, rhinoceros and antelope), all of which are frequently infected with a variety of *Trypanosoma* species, and serve as maintenance hosts for *nagana*, a persistent and fatal disease of cattle. Despite the pivotal role played by wildlife as maintenance hosts to diverse array of pathogens, these animals may be afflicted by both morbidity and mortality as a consequence of livestock borne diseases (Fynn et al., 2016). This is well illustrated by the effects of bovine tuberculosis, which was originally introduced to the African continent by cattle, and has since proliferated to a point where at least 14 species of carnivores, primates and wild ruminants are currently affected (De Garine-Wichatitsky, 2013). With that being said, the threat of disease transmission remains the greatest obstacle towards the co-existence of

livestock and wildlife populations in Africa (Bourn & Blench, 1999; Bengis et al., 2004), and may in certain cases limit the expansion of the continents agricultural sector.

In recent years, the wildlife/livestock farming model has gained significant traction amongst those involved in wildlife conservation, who have frequently advocated the method as a means of bolstering conservation efforts beyond the range of protected areas (i.e., on private land). The basis for this advocacy is underpinned by the fact that many parks exhibit an inadequate amount of area needed to sustain wildlife populations, and therefore may offer a limited capacity for the conservation of large wildlife species (Miller & Harris, 1977; Redford & Robinson, 1991). This statement is well evidenced in the South African context, where 400 protected areas constitute a mere six percent of the country's terrestrial environment (Van Schalkwyk, 2006). In addition to the aforementioned, it is believed that the conservation of wildlife on private lands enables wildlife to exploit broader ranges, and would ultimately afford these populations an opportunity to reach sizes that are conducive to their conservation, albeit that the range and its resources would be shared with domesticated stock (Western, 1989; Simonetti, 1995).

Despite the benefits of this approach for both the conservation of biodiversity and agricultural expansion, Simonetti, (1995), emphasized its inherent risks *(viz*. disease) and the potential thereof to offset the cost/benefit ratio of the strategy. Furthermore, Alonso Aguirre & Starkey, (1994), concluded that the integration of wild and domestic populations would be a "disease-mediated task", and that the practice would invariably require routine interventions to ensure the safety of these sympatric animal populations.

According to Lloyd-Smith et al., (2009), the identification of key risk factors utilised for surveillance and interventions may be improved by enhancing the role played by ecologists in disease control programmes, who offer highly accurate mathematical model outputs which could be utilized in conjunction with real time data used by clinicians. With that being said, the collaborative efforts of both public health scientists, who commonly use epidemiological procedures together with human case data, and disease-ecologists who tend to use wildlife/livestock data to model the threat of disease, are paramount in overcoming the challenges associated with strategies that seek to integrate wild and domestic animal populations (Karesh et al., 2012). Moreover, studies by Morse et al., (2012), have underlined the importance of consolidating the efforts of both disease clinicians and ecologists. The unification of objectives between the aforementioned parties would undoubtedly improve our ability to delineate the relationship between environmental change and disease dynamics, while simultaneously promoting advances in our capacity to forecast the emergence and proliferation of novel zoonoses (Morse et al., 2012).

While we cannot ignore the fact that significant strides have been made in an effort to overcome the challenges associated with disease transmission at the wildlife/livestock interface, our ability to forecast and manage disease events within these areas are currently impeded by a limited understanding of the response of bacterial communities to land use alterations. Here we revisit the Krommelboog Farm, a commercial livestock farm and wildlife conservancy, located in the Karoo Region of the Western Cape Province of South Africa. The Krommelboog Farm is currently managed by the Landmark Foundation and differs from other farms in the area due to the avant-garde farming practices that have been employed as a means of promoting both animal production and wildlife conservation. Our initial investigation at the farm took place in 2017, during which time the farms wildlife and livestock factions had begun being integrated. Since then, the majority of the physical barriers at the farm have been removed, allowing for the total integration of the aforementioned

populations. Here we assess the long-term implications of animal integration on the composition of bacterial communities across the farm, while simultaneously evaluating its effect on the frequency of pathogens at the wildlife/livestock interface. The aforementioned areas of interest would serve as the ideal criteria in assessing the efficacy of mixed animal farming, as not only an effective economic tool, but a more sustainable model for agriculture in the Karoo region of the Western Cape Province of South Africa.

With that being said, the primary directives of this study are aimed at:

1. Delineating the bacterial communities present within soils at the Krommelboog Farm. This includes the detection, identification and quantification bacterial communities within samples across both segregated and integrated scenarios (γ-diversity), through the employment and application of NGS technologies and appropriate *in silico* treatments.
2. Elucidating and quantifying the bacterial communities within sampled areas and contrasting them against one another (α-diversity), while simultaneously highlighting core contributing phyla and patterns in bacterial community structure.
3. Establishing intra-scenario bacterial community composition (β-diversity).
4. Screening the environment for pathogenic strains of bacteria that have the capacity to economic and health losses at the Krommelboog Farm.
5. Assessing long term impact of wildlife and livestock animal integration on the density and diversity of bacterial communities at the Krommelboog Farm.

3.2 Materials and Methods

3.2.1 Sample Collection

Soil and dung (sourced from wildlife and livestock) samples were collected at the Krommelboog Farm (Fig 3.1) during May 2017 (segregated) and May 2019 (integrated) following the first winter rainfall. While both "segregated" and "integrated" samples were collected within the same spatial range, individual collection sites varied between 2017 and 2019, as our sampling effort needed to take place in areas demonstrating the highest levels of animal activity . For the purpose of this study, a total of 10 sites within the Krommelboog Farm were identified and selected. Across these sites, a total of 16 samples were harvested. Nine of these samples were collected in the form of soil, while the remaining seven samples were collected in the form of dung. It was imperative that the collected samples exhibited high moisture content, thereby ensuring retention of bacterial DNA in our samples. Upon being collected, all samples were processed and stored following identical treatments described in Chapter 2.2.1. A full list of these samples, their particulars and corresponding site information may be found in Table: 3.1.

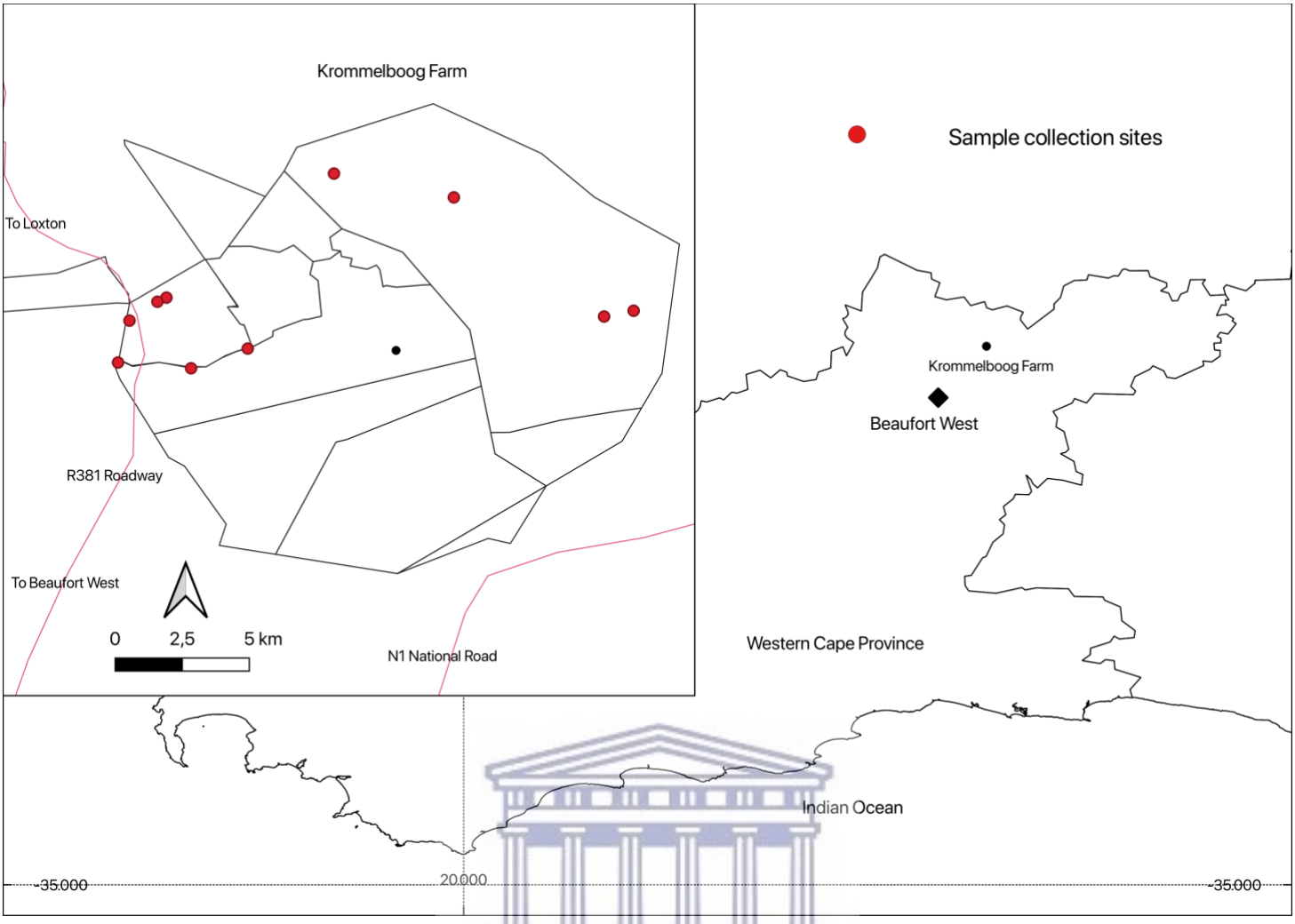

Figure 3.1: A map depicting various sites across the Krommelboog Farm, Beaufort West, South Africa, at which "integrated" soil and dung (sourced from wildlife and livestock) samples were collected in 2019. A graphical representation for "segregated" data collected in 2017 may be found in Chapter 2.

Table 3.1: A summary of "integrated" and "segregated" sites at which soil and dung (sourced from wild and livestock) samples were collected at the Krommelboog Farm, Beaufort West

Scenario	Field Name	Sample Type	Coordinates	Lab Code
Integrated	Kambro Dam	Soil	-32.052600, 22.769133	YB.12
Integrated	Kambro Dam	Dung	-32.052600, 22.769133	YB.4
Integrated	Welgevonden Ent.	Dung	-32.074286, 22.752783	YB.1
Integrated	Kambro Ent.	Soil	-32.060339, 22.756626	YB.13
Integrated	Kambro Pond	Soil	-32.054083, 22.766783	YB.5
Integrated	L.England H.Rivers	Soil	-32.019200, 22.865200	YB.3
Integrated	L.England H.Rivers	Dung	-32.019200, 22.865200	YB.2
Integrated	L. England Dam	Soil	-32.011233, 22.825233	YB.6
Integrated	L. England Dam	Dung	-32.011233, 22.825233	Y.14
Integrated	Welgevonden WP 1	Soil	-32.076417, 22.777200	YB.8
Integrated	Welgevonden WP 1	Dung	-32.076417, 22.777200	YB.10
Integrated	Welgevonden WP 2	Soil	-32.069917, 22.796200	YB.15
Integrated	Krommel Main R.Bed U	Soil	-32.059033, 22.915533	YB.11
Integrated	Krommel Main R.Bed U	Dung	-32.059033, 22.915533	YB.16
Integrated	Krommel Main R.Bed L	Soil	-32.057283, 22.925531	YB.9
Integrated	Krommel Main R.Bed L	Dung	-32.057283, 22.925531	YB.7
Segregated	PK 23	Dung	Unavailable	J.82
Segregated	DH 21	Dung	Unavailable	J.86
Segregated	DH 22	Soil	Unavailable	J.87
Segregated	DH 23	Dung	Unavailable	J.88
Segregated	NOOITHUIS 2	Dung	Unavailable	J.89
Segregated	NOOITHUIS 1	Dung	Unavailable	J.90
Segregated	SALMAN DAM	Dung	Unavailable	J.93
Segregated	WP 41	Soil	Unavailable	J.95
Segregated	WP 43	Soil	Unavailable	J.96
Segregated	HOUSE WP 1.1	Soil	Unavailable	J.98

3.2.2 DNA extraction

DNA was extracted from each of the 16 “integrated” samples using the same approach employed in Chapter 2.2.2.

3.2.3 V3-V4 amplification, clean up and library preparation

V3-V4 amplification, clean up and library preparation followed the same method used in Chapter 2.2.3.

3.2.4 Metagenomic data importation and mapping file generation

For this study, the 16S rRNA amplicon sequences collected for this study, were combined with sequences derived from the previous study for downstream analysis. These included all samples that had been collected from areas that were exclusively occupied by either wildlife or livestock factions. Collectively, these samples served as baseline data (non-integrated bacterial communities), against which the samples collected for this chapter (integrated bacterial communities) may be compared and contrasted. The treatment of FASTQ (raw reads) as well as the generation and validation of the mapping file needed for our analysis followed the same methods described in Chapter 2.2.4. The validated mapping file generated for the purpose of this analysis may be found in Appendix: 5.

3.2.5 Sequence consolidation, quality control and OTU assignment

Following the generation and validation of the mapping file, pair ended sequences (FASTQ) for each of the 26 samples were consolidated with PANDASeq, following the same parameters used in Chapter

2.2.5. As was the case in the previous analysis, each of the adjoined sequences were individually assessed for quality (read counts) using the Mothur protocol (Schloss et al., 2009), and information pertaining to the measures of central tendency for the dataset documented, and later used to establish the sampling depth of the analysis. OTU's were then assigned to the sequences using an open reference OTU picking strategy, and subsequently clustered at a 90.0% similarity threshold and thereafter, aligned to the SILVA 132 16S rRNA gene reference database using PYNAST (Caporaso et al., 2009; McDonald et al., 2012). The OTU clusters generated during the OTU picking process were the delineated into phylotypes, that were then contrasted against the SILVA 16S rRNA gene reference database, and classified according to various taxonomic rankings. Based on the average read count across all samples, sequences were rarefied to a depth of 5,000 reads, and a phylogenetic tree generated using the multiple sequences aligned during the OTU picking process.

The application of this method resulted in the generation of bacterial taxaplots for the two focus areas (segregated vs. integrated) for this component of our study. Datasets derived from these taxaplots followed the same transformation treatment described in Chapter 2.2.5.

3.2.6 Statistical analyses of inter-scenario bacterial communities

Refer to Chapter 2.3.6 for a detailed account of all statistical treatments employed. The methods utilized here are identical aside from the fact that this analysis employed the use of a non-parametric Student's t-test, the outputs of which were adjusted using the Bonferroni correction.

3.2.7 Pathogen Screening

Pathogen screening was achieved using the same methods described in Chapter: 2.2.7.

3.3 Results

3.3.1 Delineation of bacterial γ-diversity at the Krommelboog Farm (Segregated & Integrated)

Our analysis of soil and dung samples collected prior to, and following the integration of wildlife and livestock factions at the Krommelboog Farm, Beaufort West, rendered a total of 2,443,444 sequences across all 26 of the samples collected and analysed. Sequence counts for our samples ranged from 277,582 to 51. The sample exhibiting the greatest number of sequences was sample YB.14 (N=277,582), which was derived from a dung sample that had been collected at the farm following the integration of its' wildlife and livestock populations. The sample exhibiting the fewest sequences was sample J.86 (N=51), which was collected from dung sourced at the farm, prior to the integration of wildlife and livestock integration. Our samples returned an average of 93,978 reads with a standard deviation of 75,645.346. As was the case in the previous chapter, the low read sequence count exhibited by sample J.86, was be attributed to amplification failure, which alludes to poor sample quality, and as such was excluded from downstream analysis. Finally, it is worth mentioning that the SILVA 16S rRNA database was incapable of assigning classifications to 1.6% of the taxa detected by our analysis, and this included 2.6% of the taxa detected from "integrated" samples and 0.6% of the taxa detected from "segregated samples.

3.3.1.1 Core microbiome taxa identified across all sampled scenarios

Core microbiome taxa (phylum-level) identified within "integrated" and "segregated" areas across the Krommelboog Farm.

Our investigation of bacterial γ-diversity of soil and dung samples collected prior to and following the integration of wildlife and livestock factions at the Krommelboog Farm resulted in the detection 38 bacterial phyla (Fig: 3.2). The core phyla included: Planctomycetes (30.8%), Firmicutes (13.4%),

Actinobacteria (13.1%), Bacteroidetes (8.5%), Proteobacteria (7.1%), Verrucomicrobia (6.5%), Acidobacteria (3.9%), Chloroflexi (3.7%), Euryarchaeota (3.2%), and the candidate phylum TM7 (3.2%). A comprehensive summary of all classified bacterial phyla, and their respective abundances may be found in Appendix: 6.

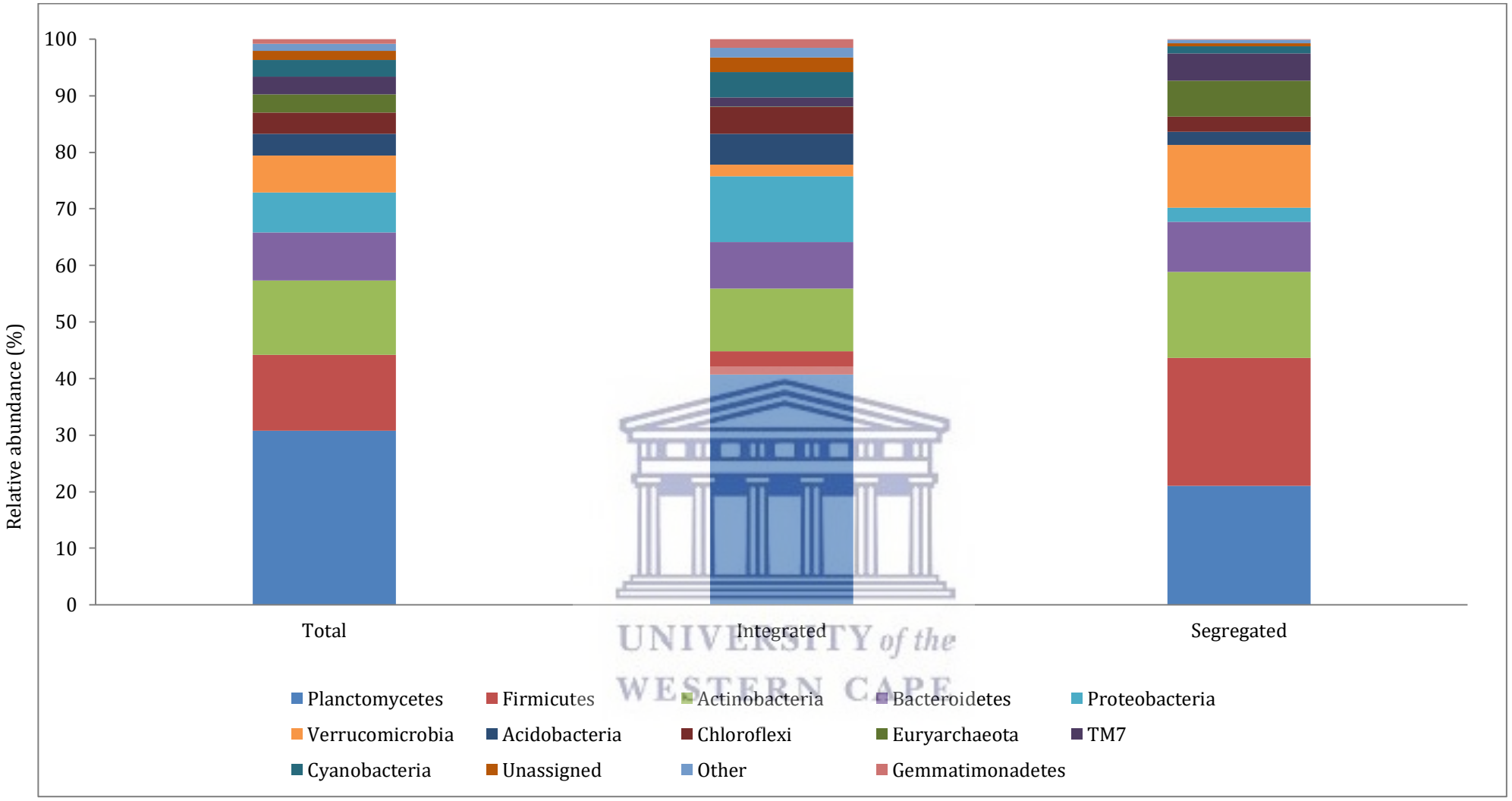

Figure 3.2: Filtered taxaplots (Integrated vs. Segregated) illustrating the (phylum-level) relative abundance of bacterial taxa detected and classified using the 16S rRNA gene.

Core microbiome taxa (family-level) identified within "integrated" and "segregated" areas across the Krommelboog Farm.

At the rank of family, the phylotypes detected and classified from both "integrated" and "segregated" areas of the Krommelboog Farm, collectively returned a total of 343 bacterial families (Fig: 3.3), with partially classified taxa accounting for 44.0% of all the bacterial families detected within the study area. The core taxa detected across "integrated" and "segregated" areas of the Krommelboog Farm included: *Micrococcaceae* (8.8%), *Verrucomicrobiaceae* (5.9%), *Ruminococcaceae* (5.4%), *Gemmataceae* (4.2%), *Sphingomonadaceae* (3.3%), *Methanobacteriaceae* (3.2%), *Ellin6075* (3.1%), *Clostridiaceae* (2.5%), *Lachnospiraceae* (2.4%) and Isophaeraceae (2.3%). A comprehensive summary of all classified bacterial families, and their respective abundances may be found in Appendix: 7.

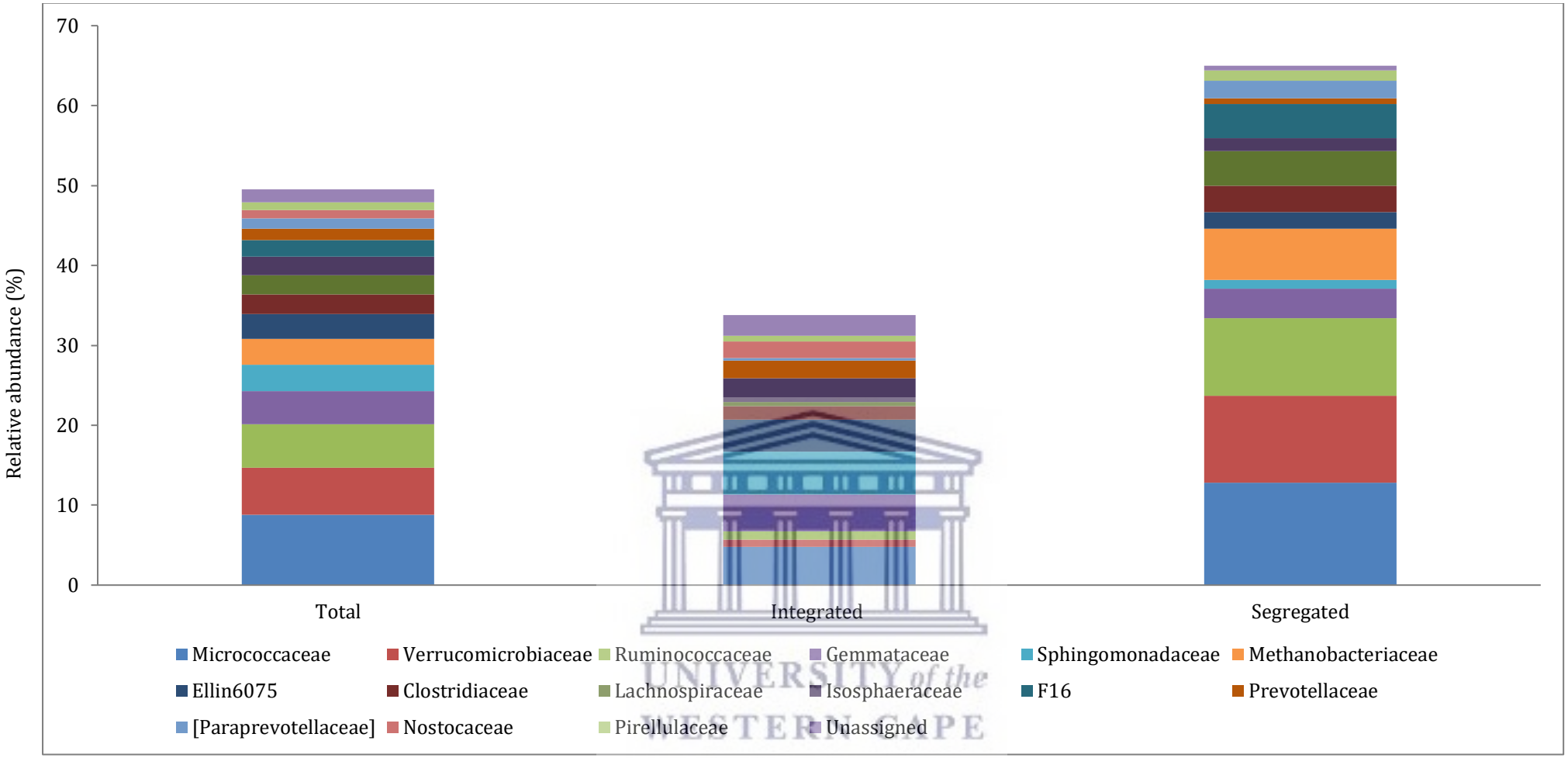

Figure 3.3: Filtered taxaplots illustrating the (family-level) relative abundance of bacterial taxa detected and classified using the 16S rRNA gene. Partially classified families and families with low representation (<0.1%) have been omitted (Total = 50.5%; Integrated = 66.2% and Segregated = 35.0%).

Core microbiome taxa (genus-level) identified within "integrated" and "segregated" areas across the Krommelboog Farm.

Genus level classification of detected taxa resulted in our phylotypes collectively returning a total of 579 bacterial genera (Fig: 3.4), with partially classified taxa accounting for 66.83% of all the bacterial genera detected within the study area. The core bacterial genera included: *Arthrobacter* (8.5%), *Akkermansia* (5.8%), *Gemmata* (4.0%), *Methanosphaera* (2.3%), *Prevotella* (1.4%), *Clostridium* (1.2%), *Kalistobacter* (1.2%), *Sphingomonas* (1.1%), *Methanobrevibacter* (0.9%) and *Epulopisicium* (0.8%). A comprehensive summary of all classified bacterial genera, and their respective abundances may be found in Appendix: 8.

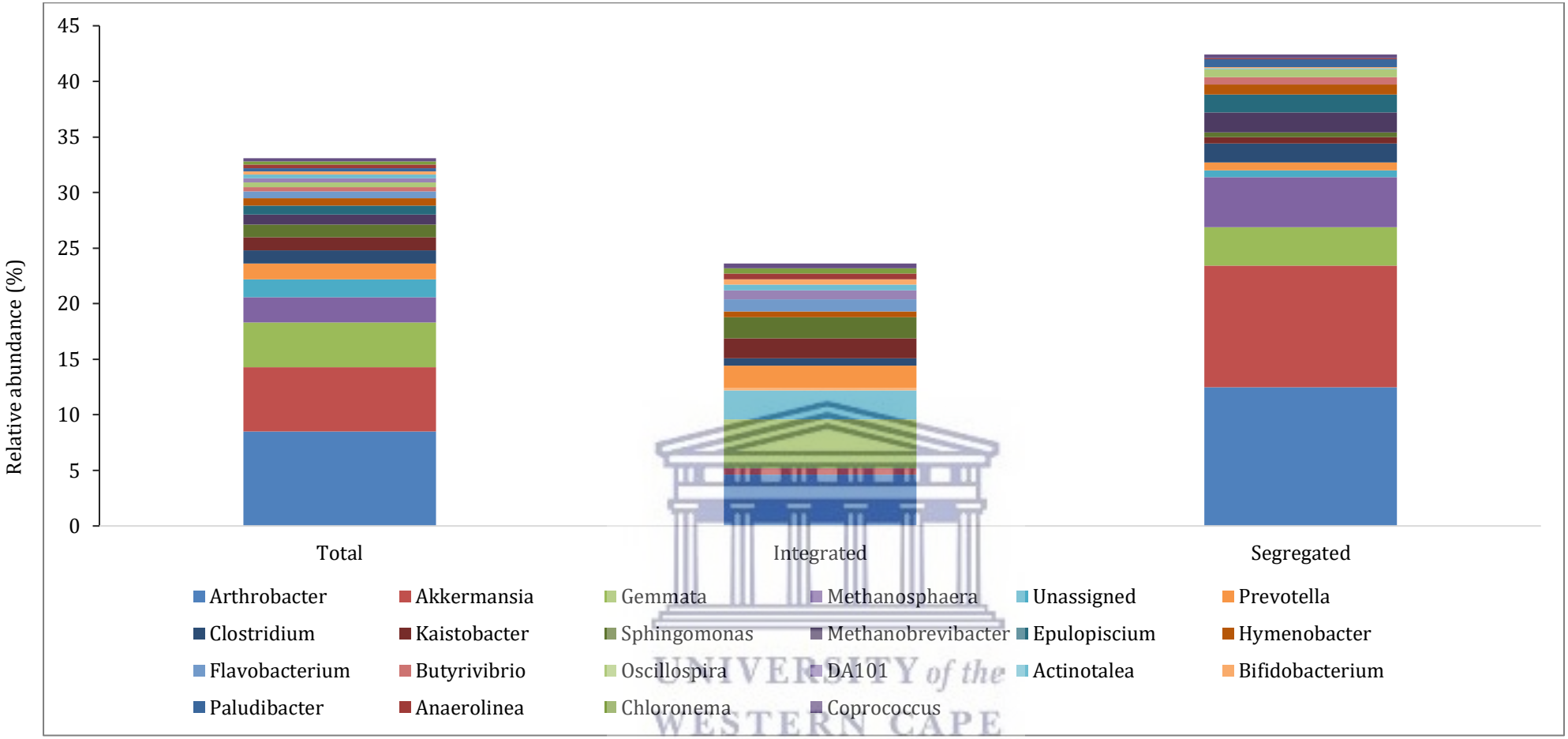

Figure 3.4: Filtered taxaplots illustrating genus-level bacterial abundance for taxa detected and classified using the 16S rRNA gene. Partially classified genera and genera with low representation (>0.1%) have been omitted (Total = 66.9%, Integrated = 76.4% and Segregated = 57.6%).

3.3.2 Delineating *A* and *B*-diversity at the Krommelboog Farm

3.3.2.1 Core microbiome taxa (phylum-level) identified by scenario type (Segregated vs. Integrated)

Our investigation into the α-diversity of bacterial assemblages at the Krommelboog farm, prior to and following the integration of wildlife and livestock factions returned the following results: Samples derived from "segregated" areas, where areas were exclusively populated by either livestock or wildlife factions, returned a total of 16 bacterial phyla, the fewest between our two sampled scenarios. The core phyla derived from "segregated" scenarios included: Firmicutes (22.7%), Planctomycetes (22.0%), Actinobacteria (15.2%), Verrucomicrobia (11.1%), Bacteroidetes (8.8%), Euryarchaeota (6.4%), TM7 (4.8%), Chloroflexi (2.7%), Proteobacteria (2.5%) and the Acidobacteria (2.3%). In contrast, samples collected from integrated" scenarios yielded a total of 18 bacterial phyla, and included the following taxa: Planctomycetes (40.7%), Proteobacteria (11.7%), Actinobacteria (11.1%), Bacteroidetes (8.2%), Acidobacteria (5.5%), Chloroflexi (4.7%), Cyanobacteria (4.5%), Firmicutes (4.1%), Verrucomicrobia (2.0%) and TM7 (1.6%).

3.3.2.2 Core microbiome taxa (family-level) identified by scenario type (Segregated vs. Integrated)

At family level, samples derived from "segregated" areas at the Krommelboog Farm, returned a total of 51 bacterial families – the fewest number of families between the two sampled scenarios. Core families comprising these samples included:

Micrococcaceae (12,8%), *Verrucomicrobiaceae* (10,9%), *Ruminococcaceae* (9.7%), *Methanobacteriaceae* (6.4%), *Lachnospiraceae* (4.3%), F16 (4.3%), *Gemmataceae* (3.7%), *Clostridaceae* (3.3%), [*Paraprevotellaceae*] (2.2%) and *Ellin6075* (2.1%). In contrast, samples derived from areas following integration, yielded a total 74 bacterial families, the core taxa of which included: *Sphingomonadaceae* (5.4%), *Micrococcaceae* (4.8%), *Gemmataceae* (4.6%), *Ellin6075* (4.0%), *Isophaeraceae* (3.0%), *Prevotellaceae* (2.2%), *Nostaceae* (2.1%), *Clostridiaceae* (1.7%), *Micromonosporaceae* (1.4%) and *Geodermatophilaceae* (1.2%). Partially classified and poorly represented (>0.1%) taxa accounted for 47.7% ("integrated") and 26.7% ("segregated") of the total number of bacterial families detected across all scenarios.

3.3.2.3 Core microbiome taxa (genus-level) identified by scenario type (segregated vs. integrated)

Genus-level taxonomic investigations revealed the following information. Samples sourced during which time wildlife and livestock factions were "segregated" yielded a total of 47 bacterial genera, the core taxa of which included: *Arthrobacter* (12.5%) *Akkermansia* (10.9%), *Methanosphaera* (4.5%), *Gemmata* (3.5%), *Methanobrevibacter* (1.8%), *Clostridium* (1.7%), *Epulopisicum* (1.6%), *Hymenobacter* (0.9%), *Prevotella* (0.7%), and *Butyvibrio* (0.7%). In contrast, samples sourced following the integration of the aforementioned animal populations ("integrated) yielded a total of 71 bacterial genera. The core taxa identified within these samples included: *Arthrobacter* (4.6%), *Gemmata* (4.4%), *Prevotella* (2.2%), *Sphingomonas* (1.9%), *Kaistobacter* (1.8%), *Flavobacterium* (1.1%), *DA101* (0.8%), *Clostridium* (0.7%), *Akkermansia* (0.6%), and *Novosphingobium* (0.6%). At genus-level, partially classified or poorly represented taxa constituted 74.0% ("integrated) and 67.2% ("segregated") of the total number of bacterial genera detected across all scenarios.

3.3.3.1 *A*-diversity rarefaction plots: Species diversity and richness metrics

Rarefaction plots generated using diversity metrics assisted in delineating the differences in bacterial species richness (Observed OTU's/ Chao1) and diversity (Shannon, PD whole tree) observed between our sampled scenarios (segregated vs. integrated) (Fig 3.5). The stabilization of our metric curves suggests an adequate sampling effort, although, the veracity of these outputs may certainly be improved with the addition of more samples for each of our scenarios.

Our indexes for species richness (Observed OTU's and Chao1) indicated a greater number of unique OTU's being detected within samples derived from "integrated" scenarios at the Krommelboog Farm. This arrangement is maintained with the Chao1 index, which considers both the observed number of unique OTU's and non-observed rare species. Similarly, differences in bacterial diversity (Shannon, PD whole tree) between "segregated" and "integrated" scenarios, indicated higher levels of observed bacterial diversity amongst samples sourced from areas in which wildlife and livestock had been integrated. Pairwise comparisons of the aforementioned scenarios using non-parametric Student's t-Test (999 Monte Carlo permutations), revealed significant differences in bacterial abundance (observed OTU's) ($p = 0.001$), species richness (Chao1) ($p = 0.001$), evenness (PD whole tree) ($p = 0.001$) and diversity (Shannon) ($p = 0.004$).

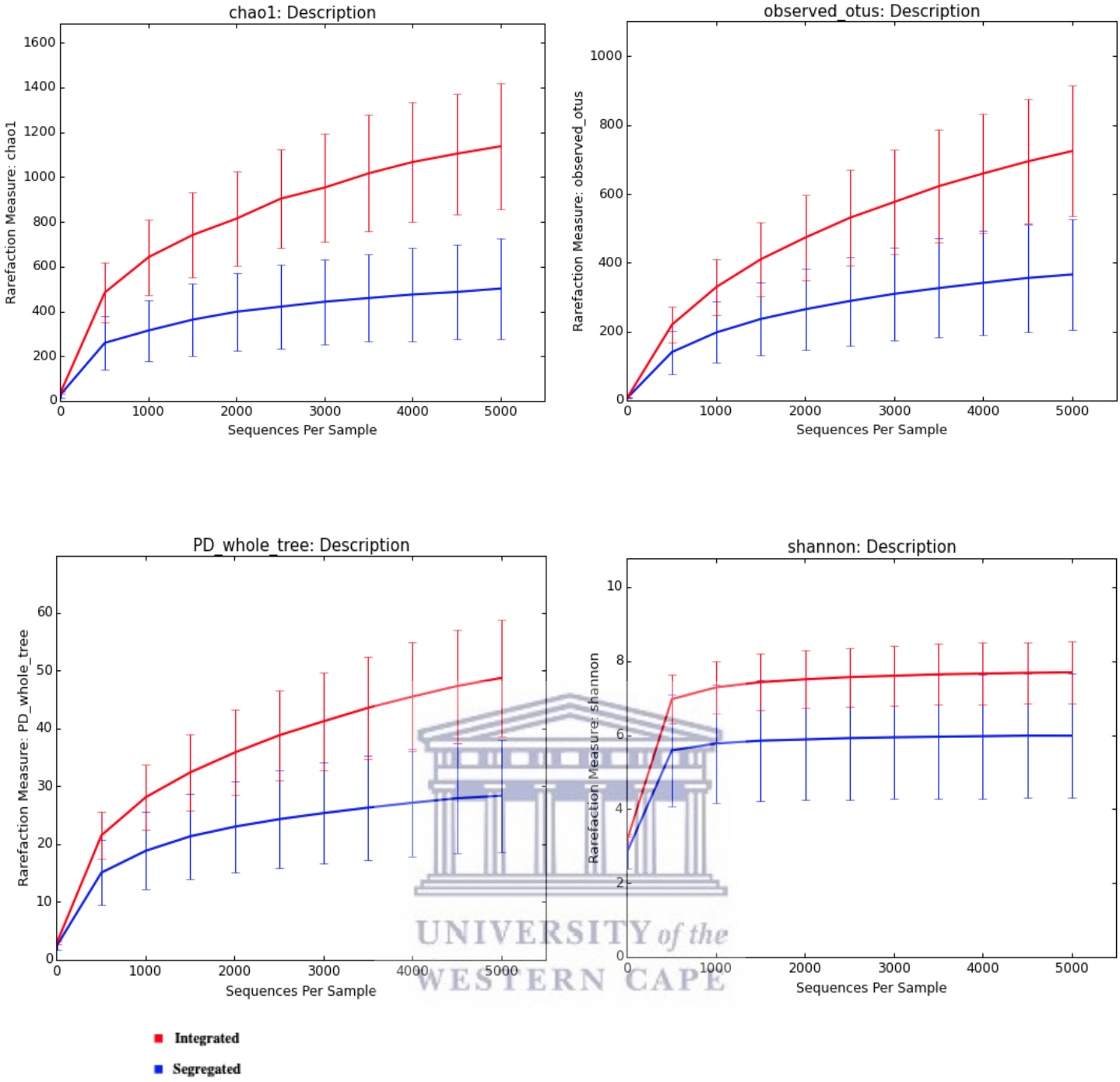

Figure 3.5: Rarefaction plots denoting differences in α-diversity between sampled scenarios at the Krommelboog Farm. The Chao1 metric (top left) estimates diversity as a measure of species abundance, Observed OTU's (top right), expresses species richness as a function of the number of operational taxonomic units (OTU's) detected across samples, PD whole tree (bottom left) estimates diversity as a function of the sum of all phylogenetic distances between detected between taxa while the Shannon metric (bottom right) estimates diversity as a function of entropy.

Table 3.2: Scenario-based, pairwise comparisons of species richness (Chao1), OTU abundance (Observed OTU's), phylogenetic diversity (PD whole tree) and diversity (Shannon) using non-parametric multiple Student's t-tests (999 Monte Carlo permutations), with Bonferroni adjusted outputs.

Chao1							
Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t-statistic	p-value
Integrated	Segregated	1137.24	280.64	502.13	224.61	5.5833	0.001
Observed OTU's							
Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Integrated	Segregated	724.62	189.67	366.03	161.86	4.5820	0.001
PD whole tree							
Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Integrated	Segregated	48.69	10.07	28.35	9.73	4.70	0.001
Shannon							
Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Integrated	Segregated	7.69	0.83	5.98	1.67	3.26	0.004

3.3.3.2 *A* and *B*-diversity: ANOSIM, NMDS and SIMPER

A-diversity: ANOSIM & NMDS

Rarefied OTU data from each of our sampled scenarios was analysed *in silico*, using a one-way analysis of similarities (ANOSIM) employing the use of the Bray-Curtis similarity index. Our analysis of phylum-level OTU composition for samples collected from both "segregated" and "integrated' sampling scenarios, indicated a significant statistical difference between the two aforementioned scenarios and their respective samples, with low levels of OTU overlap ($p = 0.002$, $R = 0.362$). This finding was further corroborated by NMDS, which graphically delineated sampled scenarios and their respective samples as a function of the OTU composition (Fig 3.6). The output of this effort demonstrated significant differences in OTU composition between our "segregated" and "integrated" samples. It is worth mentioning that a low level of overlap was observed amongst five of our samples, however, these differences are not significant enough to detract from the outputs generated from our analysis. In terms of OTU composition, samples J.87, J.88 and J.96 (sourced from "segregated" sites at the Krommelboog Farm), were subsequently clustered with samples collected within "integrated" areas; while samples YB.3 and YB.16 (sourced from "integrated" sites at the Krommelboog Farm) were subsequently clustered together with "segregated" samples. These discrepancies could potentially be attributed to lower bacterial loads, which may ultimately have influenced OTU composition, and the relationship of these samples with the remaining samples employed in this analysis.

In terms of α-*diversity*, our assessment of scenario specific OTU's (level = phylum) quantified the differences between our sampled scenarios at approximately 62.24%. The core phyla contributing to the phylum level OTU dissimilarity between scenarios included the following taxa: Planctomycetes

(25.12%), Firmicutes (17.21%), Actinobacteria (15.05%), Bacteroidetes (8.11%) and Proteobacteria (7.30%).

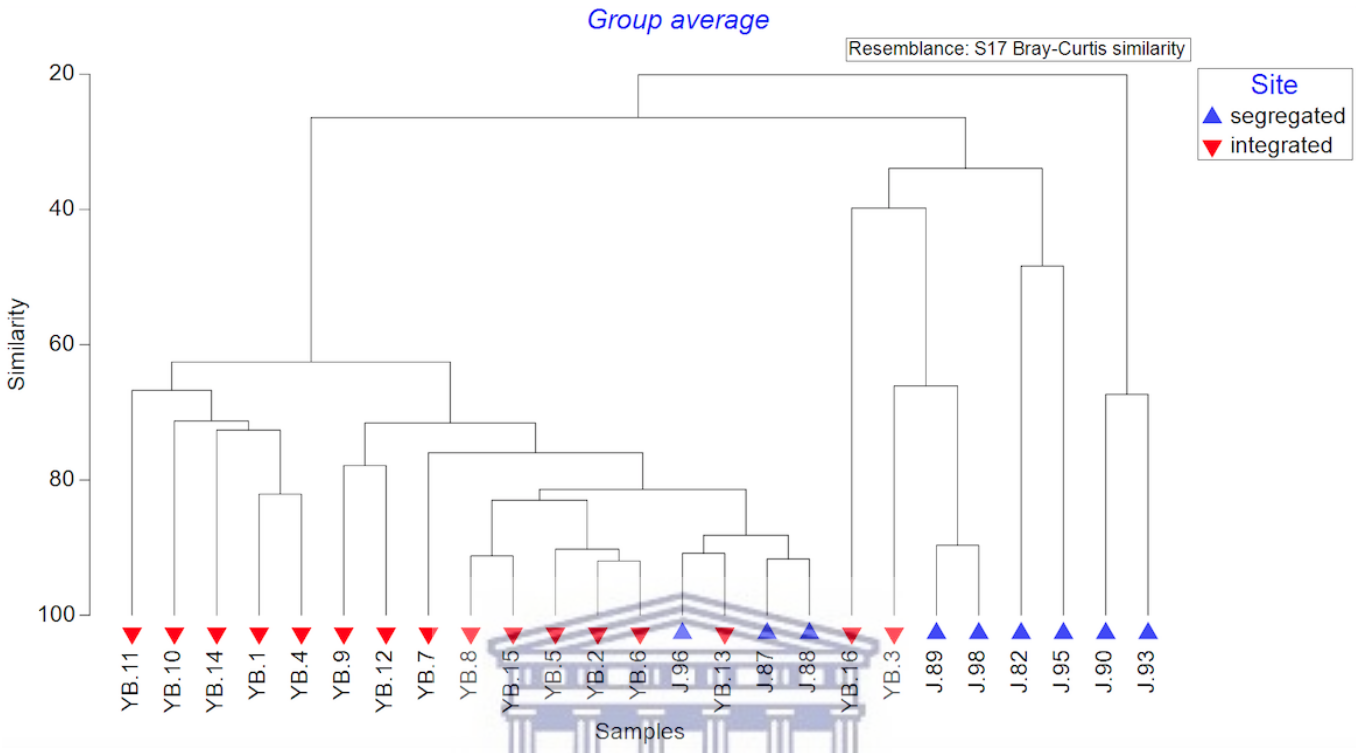

Figure 3.6: NMDS (hierarchical clustering) dendrogram of samples collected across "segregated" and "integrated" scenarios at the Krommelboog farm, in which samples were grouped according to OTU composition, using the Bray-Curtis similarity index.

B-diversity: OTU Composition

B-diversity within our "segregated" and "integrated" scenarios was calculated using a one-way SIMPER analysis, and suggested a greater OTU composition similarity between samples sourced from "integrated" samples (62.80%), while "segregated" samples demonstrated a lower levels of similarity in terms of OTU composition (29.19%).Within the "integrated" group, the high levels of OTU homogeneity observed between samples was attributed to the contributions of the following taxa: Planctomycetes (47.54%), Proteobacteria (13.04%) and the Actinobacteria (12.15%). Conversely, the low levels of OTU heterogeneity demonstrated by samples derived from the "segregated" scenario

were determined by the contributions of Planctomycetes (32.60%), Firmicutes (23.96%), Actinobacteria (12.23%) and Bacteroidetes (6.68%).

3.3.4 Detected pathogenic taxa

Our screening efforts yielded a total of 23 pathogenic bacteria across both "segregated" and "integrated" samples collected at the farm. Pathogenic taxa were identified through the consultation of the OTU table generated by our Illumina analysis, which provided us with a breakdown of bacterial prevalence and distribution across samples scenarios and their respective samples. The 23 identified pathogens constituted 4.6% of all the fully classified bacterial taxa identified at genus level (and species level, where possible), with 21 pathogens being detected within "integrated" scenarios (91.3%), and the remaining two pathogens being detected within "segregated" scenarios (8.7%). "Integrated" areas returned an average of 1.3 pathogens per sample (N=16), whereas "segregated" areas returned an average of 0.55 pathogens per sample (N =9). A full of list detected bacterial pathogens and their distributions may be found in Table: 3.3.

Table 3.3: Summary of pathogenic bacteria detected within soil and faecal samples within segregated and integrated scenarios at the Krommelboog Farm, Beaufort West.

Taxa	Scenario	Sample Type	Sample
Acinetobacter sp.	Integrated	Soil & Dung	YB1,4,5,6,7,8,9,11,12,13
Actinomycete sp.	Integrated	Soil & Dung	YB1,5,6,7,8,9,11,12,13,15
Aeromonas hydrophilia	Integrated	Soil	YB9
Anabaena sp.	Integrated	Soil	YB11
Bosea thiooxidans	Integrated	Soil & Dung	YB1,4,5,6,8,9,11,12,13,14,15
B. paraconglomeratum	Integrated	Soil	YB15
Clostridium sp.	Integrated	Soil & Dung	YB1,4,5,6,7,8,9,11,12,14,15
Cronobacter universalis	Integrated	Dung	YB7,14
Dietzia maris	Integrated	Soil & Dung	YB4,8,11
Enterobacter cloacae	Integrated	Soil & Dung	YB7,9,14
Enterococcus sp.	Integrated	Dung	YB14
Escherichia coli	Integrated	Dung & Soil	YB7,12
Gordonia terrae	Integrated	Soil	YB5,6,8
Herbaspirillum sp.	Integrated	Dung	YB7
Klebsiella pneumoniae	Integrated	Soil & Dung	YB1,6,7,11,12,15
Massilia timonae	Integrated	Soil & Dung	YB1,4,5,6,7,8,9,11,12,14,
Paenibaccilus sp.	Integrated	Soil & Dung	YB1,8
Paracoccus sp.	Integrated	Soil	YB8,11
Pseudomonas sp.	Integrated	Soil & Dung	YB1,4,5,6,7,8,912,13
Roseomonas sp.	Integrated	Soil & Dung	YB1,4,5,6,7,8,9,14,15,16
Sphingobacterium sp.	Integrated	Soil & Dung	YB5,6,16
Exiguobacterium sibiricum	Segregated	Dung	J.93
Clostridium gasigenes	Segregated	Soil	J.95

3.4 Discussion

Our assessment of environmental samples sourced at the Krommelboog Farm provided us with invaluable information relating to the farm's bacterial communities and their subsequent response to changes in agricultural practices (*viz.* the removal of physical barriers at the farm, and the subsequent integration of the farm's wildlife and livestock populations). The findings of the paper were generated through the application of NGS technology, targeting the 16S rRNA gene, and supplemented with appropriate *in silico* analyses of the sequences generated using QIIME 1.9.1 (Caporaso et al., 2010).

Our investigation culminated in the detection of 38 bacterial phyla, comprising a total of 343 families and 532 genera. The dominant bacterial phyla across all of our samples were the Planctomycetes (30.8%) and the Firmicutes (13.4%), which collectively accounted for 44.2% of all bacterial phyla detected in this study (Fig 3.2). This observed dominance at phylum level was carried over to lower levels of classification. While our investigation was capable of detecting a total of 343 bacterial families, we find that our samples were dominated by seven bacterial families, namely, Micrococcaceae (12.8%), Verrucomicrobiaceae (10.9%), Ruminococcaceae (9.7%), Methanobacteriaceae (6.4%), Lachnospiraceae (4.3%), f16 (4.3%) and Gemmataceae (3.7%). While these bacterial families constituted only 2.04% of the total number of families detected, their contributions to family level OTU's detected are in excess of 50.0% (Fig 3.3). Similarly, genus-level taxaplots were dominated by eight bacterial genera, namely, *Arthrobacter* (8.5%), *Akkermansia* (5.8%), *Gemmata* (4.0%), *Methanosphaera* (2.3%), *Prevotella* (1.4%), *Clostridium* (1.2%), *Kalistobacter* (1.2%), *Sphingomonas* (1.1%). These families represented only 1.3% of the families detected, but contributed 25.0% in terms of overall abundance (Fig 3.4).

3.4.1 Krommelboog bacterial community composition: *A*-diversity & *B*-diversity

Our analysis provided us with indispensable information relating to the arrangement of soil bacterial communities during periods of segregation, and their response following the integration of the farm's animal populations. *A*-diversity investigations into bacterial communities, offered us a better understanding of scenario specific differences in bacterial community composition at the Krommelboog Farm. *A*-diversity rarefaction plots (Fig 3.5), indicated an adequate sampling effort, as evidenced by the stabilization of our various curves, which suggests that the sequences generated by this analysis serve as an accurate representation of the bacterial identities with sampled scenarios. Multiple Student's t-tests performed on sequences indicated a significant difference in both species' diversity (Shannon, PD whole tree) and species richness (Chao1, Observed OTU's) between sampled scenarios, with "integrated" samples registering higher levels of diversity (Table: 3.2). This was corroborated by NMDS, which showed low levels of OTU overlap between our samples ($p = 0.002$, $R = 0.362$) (Fig 3.6). Our β-diversity assessment of scenario specific sequences showed a greater level of OTU similarity within "integrated" samples (62.80%), and lower levels of OTU similarity between "segregated" samples (29.19%).

The results of our α-diversity analysis shows that soils across the Krommelboog Farm continues to be dominated by two bacterial phyla, namely, Planctomycetes and Firmicutes, which is well-illustrated by the taxaplots generated by our analysis (Fig 3.2). It is evident that the bacterial communities within soils sampled across the farm have fluctuated since our preliminary investigation. Planctomycetes now holds the greatest relative abundance of all detected bacterial phyla, and the soil bacterial community within "integrated" samples now bear a greater resemblance to the "wildlife" samples analysed in Chapter: 2. Considering studies by Buckley & Schmidt, (2003), which offered evidence to suggest that Planctomycetes populations may be correlated with soil disturbance, we can conclude that the

level of animal-induced soil disturbance has decreased at the Krommelboog Farm. Declines in soil disturbance at the farm, have also led to declines in the Firmicutes and its members, which typically thrive in highly disturbed environments due to their endospore-forming capabilities (Nicholson et al., 2002; Yung, 2006).

In order to better understand this claim, we need only look at the spatial distribution of animals at the farm during the period of segregation, and contrast it against the current arrangement at the Krommelboog Farm, where animals have been integrated.

When we undertook our initial investigation in 2017, the total area available for animal occupation at the farm was limited by fences. These fenced off sections (i.e., shepherd camps), meant that animals (livestock in particular), registered high stocking rates within these areas, which has been shown to be directly correlated with soil disturbance (Dubeux & Sollenberger, 2020). While livestock were routinely rotated between shepherd camps, the limited number of camps meant that the short interval between occupation and reoccupation would have maintained soil disturbances within these areas, and hampered the recovery of affected microbiota.

By 2019, during which the samples for this study had been collected, the vast majority of physical barriers at the farm had already been removed, and a sufficient amount of time had elapsed to allow for the majority of the farm's animal populations to fully integrate. The removal of physical barriers, and the subsequent integration ultimately increased the total area allocated for the occupation by livestock, greatly reduced the stocking rate of animals at the Krommelboog farm. Since the stocking rate of animals is directly correlated with the degree of animal induced soil disturbance (Dubeux & Sollenberger, 2020), we postulate that the observed increases in soil bacterial diversity at the farm may be attributed to the integration – which has greatly reduced the physical impact of animals on

soils (Fleischner, 1994; Belsky & Gelbard, 2000). With that being said, a clearer understanding of the soil microbial communities at the Krommelboog Farm could be achieved through the inclusion of both rainfall and temperature data – both of which play an important role in shaping soil microbial communities.

3.4.2 Pathogen Screening

Our consultation of the OTU table generated during our 16S rRNA analysis led to the detection of 26 pathogenic strains of bacteria, 91.3% of which were detected within the "integrated" scenario, while the remaining 8.7% were detected within the "segregated" scenario. These results echo the concerns of Bengis, Kock & Fischer, (2002), who emphasized the elevated risk of bi-directional pathogen transmission at the wildlife/livestock interface. Furthermore, the high levels of pathogens detected in soil and dung samples at the Krommelboog Farm following the integration of its animal populations, and emphasizes the concerns raised by Bester & Penzhorn, (2002), who postulated an increased probability of pathogenesis at the interfaces shared between wild and domestic animal populations. Despite the aforementioned evidence, which highlights the contribution of animal integration on the elevation of both pathogen presence and risk of disease transmission, there are other factors that need to be considered.

Firstly, natural environments and their components (e.g., soils, animals, water-bodies) are complex systems, that host a variety of pathogenic and non-pathogenic bacteria (Loynachan, 2013), many of which are unlikely to pose a threat to animal or human health. Thus, the presence of pathogens within a given environment may not be enough to constitute a threat of pathogenesis. According to Keesing et al., (2010), pathogenesis typically occurs as a consequence to changes within environments, where these changes culminate in a reduction in the biological diversity of organisms within their hosting

ecosystems. Furthermore, Keesing et al., (2010), argued that there is mounting evidence, which suggests that the incidence of disease transmission is inversely proportional to the biological diversity within any given ecosystem (Keesing et al., 2010). The results of this investigation have demonstrated that elevated levels of bacterial community diversity (Table 3.2) – a consequence of animal integration – has invariably led to a greater diversity of potential pathogens within our samples (Table: 3.3). In contrast, the arrangement at the farm prior to integration, yielded lower levels of diversity (Table 3.2), and a correspondingly lower number of pathogens being detected, although their individual contributions to bacterial community composition were higher (Table: 3.3). However, as previously stated, the number of pathogens detected, and their contributions to the total number of unique OTU's identified in the study, may not be enough to draw any definitive conclusions about the actual threat of disease transmission at the Krommelboog Farm. For one to accurately quantify the threat of pathogenesis, the OTU count for each pathogen, and the collective contribution of all pathogenic OTU's to the total number of OTU's detected in the analysis would also need to be considered.

The OTU counts for the 23 pathogens detected at the farm following integration ranged from 5 – 1,298 (Table: 3.4), accounting for 2,639 OTU's of the 62,580 OTU's detected in this study (Total OTU's included both partially and fully classified taxa). While these pathogenic OTU's accounted for 4.6% of the total number of OTU's detected, the OTU contributions of individual pathogens were minimal. Taking the aforementioned into consideration, we postulate that the integration of animals at the Krommelboog Farm has invariably led to an increased diversity of bacterial assemblages. Moreover, this diversity manifests itself in the form of elevated number of pathogenic taxa, however, the individual OTU contributions of pathogens in relation to the total number of OTU's detected in our analysis may be too low to constitute a threat to both economic activities and animal/human health at the Krommelboog Farm. The results here, echo the sentiments of Keesing et al., (2003), that suggested that the threat of disease outbreaks within animal populations may be inversely proportional to the level of genetic diversity of organisms within said populations.

Table 3.4: Summary of the 23 pathogenic bacteria detected within “segregated” and “integrated” soil and faecal samples at the Krommelboog Farm, Beaufort Went; and their clinical implications.

Taxa	Scenario	Clinical Implications	OTU Count
Acinetobacter sp.	Integrated	Pneumonia, meningitis (Dijkshoorn, Nemec & Siefert, 2007)	1298
Actinomycete sp.	Integrated	Actinomycosis (Yeager et al., 1986)	340
Aeromonas hydrophilia	Integrated	Nosocomial pneumonia, meningitis (Bhowmick & Bhattacharjee, 2018)	24
Anabaena sp.	Integrated	Cyanobacteria poisoning (Rastogi, Madamwar & Incharoensakdi, 2015)	8
Bosea thiooxidans	Integrated	Bacteraemia, CLABSI (Skipper, Ferrieri & Cavert, 2020)	96
B. paraconglomeratum	Integrated	Ocular infection (Murata et al., 2020)	5
Clostridium sp.	Integrated	Various animal diseases (Malone, 2004)	491
Cronobacter universalis	Integrated	Neonatal necrotizing enterocolitis, bacteraemia (Bowen & Braden, 2006)	8
Dietzia maris	Integrated	Bacteraemia, sepsis (Bemer-Melchior et al., 1999)	10
Enterobacter cloacae	Integrated	Nosocomial infections (Davin-Regli, 2015)	7
Enterococcus sp.	Integrated	Various (Selleck, Van Tyne & Gilmore, 2019)	5
Escherichia coli	Integrated	Enteric/diarrheal disease, UTI’s, sepsis (Kaper, Nataro & Mobley, 2004)	41
Gordonia terrae	Integrated	Cutaneous infections (Blanc et al., 2007)	18
Herbaspirillum sp.	Integrated	Bacteraemia (Dhital et al., 2020)	5
Klebsiella pneumoniae	Integrated	Meningitis, Necrotizing fasciitis (Siu et al., Struve et al., 2015)	29
Massilia timonae	Integrated	Lymphadenopathy (Van Craenenbrock et al., 2011)	21
Paenibaccilus sp.	Integrated	Clinical infections (Clermont et al., 2015)	6
Paracoccus sp.	Integrated	Includes *P. yeei*, assoc. with human pathogenesis (Daneshvar, 2003)	6
Pseudomonas sp.	Integrated	Bacteraemia, pneumonia, soft tissue infection (Gellatly & Hancock, 2013)	187
Roseomonas sp.	Integrated	Bacteraemia (Gladstone et al. 2011)	8
Sphingobacterium sp.	Integrated	Includes *S. multivorum* and *S. spiritivorum*, bacteraemia (Tronel et al., 2003)	26
E. sibiricum	Segregated	Anthrax-like cutaneous lesions (Tena et al., 2014)	n/a
Clostridium gasigenes	Segregated	Vacuum-packed meat spoilage, food poisoning (Esteves et al., 2022)	n/a

3.5 Conclusion

Our investigation at the Krommelboog Farm, was directed at assessing the long-term impacts of wildlife and livestock animal integration on the soil bacterial compositions, using appropriate high-throughput amplicon sequencing technologies and *in silico* treatments. Our analysis of γ-diversity offered us insights into bacterial community structure, and aided in identifying the most well represented taxa across all of our samples.. In addition to this, our statistical analysis comparing the bacterial diversity of "integrated" and "segregated" areas the farm, showed a significant difference in the species richness and bacterial diversity between the sampled scenarios, with "integrated" scenarios registering higher levels of both bacterial diversity and species richness. *B*-diversity analyses indicated a greater level of OTU composition similarity between "integrated" samples (62.8%), while "segregated" samples registered a much lower OTU composition similarity (29.19%).

Observations show bacterial communities ("integrated") assuming a structure and composition similar to that found in "wildlife" areas of the farm prior to integration, the identity of which we believe bears a greater resemblance to that of soils found in undisturbed (see Chapter: 2) ecosystems. While animal integration has the potential to offset bacterial community composition and structure, this impact is largely governed by the stocking rates of these animals, rather than the introduction of microbia from animals themselves. The findings of this study lead us to believe that farming with a combination of wildlife and livestock may indeed be a sustainable form of farming. Although the initial capital investments for wildlife are higher than traditional livestock such as cattle and sheep, wildlife farming requires less maintenance (i.e., medical care in the form of routine antibiotics) and lower upkeep costs. The heterogeneity amongst the animals being farmed equates to a wider variety of niches being occupied across the area, by a fewer number of animals, thereby facilitating the recovering of vegetation and soils, and ultimately, healthier soil bacterial communities. This study has also

highlighted the effects of animal stocking rates, which, regardless of the farming strategy being employed, tends to dictate the intensity of environmental disturbances, which have the ability to offset bacterial community diversity and species richness.

In terms of pathogenesis, this study has demonstrated the potential impact that animal integration has on the prevalence and distribution of disease-causing bacteria. Consultation of OTU's generated by our analyses, indicates a high prevalence of pathogens per sample following the long-term integration of animals, when compared to samples sourced from segregated samples. The prevalence of these pathogens however, appear to be a consequence of elevated levels of bacterial diversity, which is to be expected. Moreover, the OTU counts corresponding to detected pathogens are relatively low, and thus unlikely to pose an immediate threat to the health of animals and humans at the Krommelboog Farm, and even less likely to constitute a threat to its economic activities. With that being said, we believe that the method of farming currently being employed by the Landmark Foundation, at the Krommelboog Farm, Beaufort West, has the potential to benefit farmers in the immediate are by bolstering productivity, and thereby reducing the impact of economic challenges that are associated with resource scarcity. Furthermore, the diversification of animals within an animal farming system, offers farmers the opportunity to expand operations at their farms, thereby generating multiple streams of revenue. Lastly, based on the response of soil bacterial communities, we can conclude that this approach is sustainable in its ability to promote diversity within soil microbiota, which may in turn serve as a mitigating factor against major disease outbreaks at these locations.

Chapter 4: Summary of conclusions and recommendations

The focus of this study was aimed at elucidating the relationship between the inclusion of wildlife into traditional livestock systems and the threat of zoonotic disease transmission at the Krommelboog Farm – a commercial livestock farm, and wildlife conservancy that is managed by the Landmark Foundation in Beaufort West, South Africa.

This study was undertaken due to a recent trend within the agricultural sector, which has seen many South African farmers abandoning conventional livestock (*viz.* cattle & sheep) in favour of wildlife, or in many cases, opting to farm with a combination of the aforementioned animals (Bengis, Kock & Fischer, 2002; Bekker, Jooste & Hoffman, 2011). The popularity of game farming within a South African context, is underpinned by its high profitability (Steyn, 2013); its ability to allow for the diversification of economic activities (i.e., ecotourism) (Bothma, 2002; Butler et al., 2005); and lower costs with regard to the daily maintenance and the upkeep of animal health (Pollock & Lit, 1969). Despite the economic benefits of the aforementioned approach, the sustainability of the practice has, in recent years been contested (Skinner, 1970; Morse, 1995; McMichael, 2004; Woolhouse & Gowtage-Sequeria, 2005; Tomley & Shirley, 2009). Perhaps the greatest threat to farmers that have adopted the aforementioned method, is that wildlife populations are known to harbour a variety zoonotic-disease causing pathogens, which pose a serious threat to animal/human health and economic activities at these farms (Morse, 1995; McMichael, 2004; Woolhouse & Gowtage-Sequeria, 2005; Tomley & Shirley, 2009). Complicating matters even further, Bester & Penzhorn, (2002), have postulated that the potential for disease transmission is further elevated where wild and domestic animal populations have been integrated.

Here we elucidated bacterial communities present within soils and animals, through the application of NGS high-throughput sequencing technologies, which offered us insights relating to the relationship between mixed animal farming, and bacterial community ecology. Soil and dung (sourced from wildlife and livestock) samples were analysed assessed in terms of their bacterial composition, and screened for the presence of pathogens that may pose a threat to animal and human health and economic activities within the study area.

Our preliminary investigation (see Chapter: 2) of bacterial communities present within soil and dung samples within "wildlife", "livestock", and "contact" regions of the Krommelboog Farm in 2017, provided us with invaluable insights into bacterial community composition (density, diversity and evenness). Our analysis of sequences generated from soil and dung samples culminated in the detection of 38 bacterial phyla, comprising a total of 380 families and 532 genera. The dominant bacterial phyla across all of our samples included the Firmicutes (28.0%) and Planctomycetes (19.8%), which collectively accounted for 47.8% of bacterial phyla detected in this study (Fig 2.2). Furthermore, our α-diversity elucidation of bacterial samples showed distinct bacterial communities within samples from each of our sampled scenarios, however, statistical analysis of OTU's indicated no significant differences in either bacterial diversity and species richness between these scenarios. Furthermore, our analysis of intra-scenario (β-diversity) bacterial community composition indicated that "contact" scenarios demonstrated the highest levels of OTU similarity between taxa, while "livestock" scenarios demonstrated the lowest levels of intra-scenario OTU similarity among the three sampled scenarios.

The results of our α-diversity analysis indicated that the sampled areas were dominated by two suites of bacteria, namely, the Planctomycetes, which were more prevalent within "wildlife" scenarios, and the Firmicutes, which dominated both the "livestock" and "contact" scenarios. Furthermore, our results showed the total displacement of Planctomycetes and its members within scenarios that were

dominated by the Firmicutes. A consultation of the available literature offered us insights into the observed community composition of the three sampled scenarios at the Krommelboog Farm. Firstly, studies by Buckley & Schmidt, (2003), in which the dynamics and diversity of bacterial communities with agricultural ecosystems were investigated, provided evidence that suggested that Planctomycetes abundance may be correlated with soil disturbance. This claim was further corroborated by Buckley et al., (2006), where Planctomycetes prevalence was found to be inversely proportional to the level of disturbance within soil. Based on these findings and our observations relating to bacterial community composition, we postulated that the" livestock" and "contact" scenarios demonstrate higher levels of soil disturbance when compared to soils within the farm's "wildlife" areas. The prevalence of Firmicutes assemblages within "livestock" and "contact" areas at the farm were attributed to the underlying physiology of the Firmicutes and its members. Based on studied by Logan & De Vos, (2009), the Firmicutes (classes: *Bacilli* and *Clostridia*) are characterised by their endospore forming abilities. Furthermore, studies suggest that this ability offers members of the aforementioned taxonomic classes and improved survival advantage over less resilient varieties of bacteria (Nicholson et al., 2002), and offers endospore forming Firmicutes the opportunity to persist in highly disturbed environments where other bacterial suites have been supressed (Onyenwoke et al., 2004; Schimel, Balser & Wallenstein, 2007). Our assessment of farming practices and the bacterial communities at the Krommelboog Farm suggests that bacterial diversity is largely governed by the stocking rate of animals across our sampled scenarios.

During our follow up study in 2019, the vast majority of physical barriers (i.e., fences) segregating animal populations at the farm had been removed. The removal of these fences had allowed for the complete integration of wildlife and livestock factions at the Krommelboog Farm. Our analysis led to the detection 38 bacterial phyla, comprising a total of 343 families and 532 genera. The dominant bacterial phyla across all of our samples were the Planctomycetes (30.8%) and the Firmicutes (13.4%),

which collectively accounted for 44.2% of all bacterial phyla detected in this study (Fig 3.2). In addition to this, our α-diversity analysis of “integrated" and “segregated” samples indicated a significant difference in both bacterial diversity and species richness between these scenarios, with the “integrated” areas demonstrating significantly higher levels of bacterial abundance, diversity and evenness. In respect of our β-diversity analysis, we found “segregated” areas to demonstrate a higher level of intra-scenario OTU similarity when contrasted against “integrated” areas. A closer inspection of bacterial community composition identified the Planctomycetes as the greatest contributor to bacterial communities within samples collected across “segregated” areas of the Krommelboog Farm. The proliferation of Planctomycetes and its members, may be attributed to declines in animal-induced soil disturbance, as studies have shown that Planctomycetes population sizes are inversely proportional to the level of disturbance in soils (Buckley & Schmidt, 2006)

In order to better understand the declines in soil disturbance at the farm, we need to consider the spatial distribution and stocking rate of animals at the Krommelboog Farm, prior to and following animal integration. During the segregation of animal populations at the Krommelboog farm, livestock enjoyed a greater area for occupation, when compared to wildlife (see Fig 2.1). However, the rotation of herds between shepherd camps, invariably meant that livestock occupied smaller areas at any given period, thereby increasing the stocking rates and soil disturbance within their areas. In contrast, wildlife, were afforded smaller areas to occupy, the small population (~380 head) size meant that these animals registered a stocking rate that was considerably lower to that of livestock. Stocking rates were further exacerbated within contact areas at the farm, as only a small portion of the total area was being allocated towards livestock occupation, despite it being occupied by both wildlife and livestock. In contrast, the stocking rate of both wildlife and livestock decreased considerably following the complete integration of animals at the farm, as the total area allocated towards animal occupation was dramatically increased. While livestock continued to be housed and relocated between shepherd

camps, the number of shepherd camps allocated to livestock have increased following integration, thereby increasing the rotational period between shepherd camp occupation and reoccupation. While there may be more factors at play, the aforementioned factors appear to be the most obvious contributor towards the observed increases in bacterial density and diversity, following the integration of animal populations at the Krommelboog Farm.

In terms of our efforts at screening our samples for the presence of pathogens, our investigation led to the detection of 23 pathogenic strains of bacteria. These pathogens constituted 4.6% of all fully classified genera, the majority of which were detected within our "integrated" scenarios (91.3%), while the remaining 8.7% were detected within "segregated" scenarios. While the number of pathogens detected in each area alone, might suggest that animal integration carries a greater threat of pathogenesis, it is important to remember that the individual OTU counts for each of the detected pathogens contributed very little to the overall OTU count. It is also important to remember that environments naturally host a variety of pathogenic bacteria (Loynachan, 2013), many of which carry little to no threat to animals or activities within these locales. Furthermore, studies by Keesing et al., (2010) suggested that pathogenesis is more likely to occur within ecosystems that have been subjected to high levels of disturbance/transformation, where these changes would culminate in a reduction in biological diversity of bacterial communities. While animal populations have been integrated at the Krommelboog Farm, an act which may regarded as an environmental disturbance, the collective wildlife/livestock population now exhibits a broader genetic diversity, a feature that Springbett et al., (2002) considers to be a mitigating factor against disease outbreaks. With that being said, higher levels of animal diversity, coupled with lower stocking rates per unit area, have culminated in elevated levels of diversity and species richness amongst soil bacterial communities, which may potentially reduce the probability of large disease epidemics at the farm (Keesing et al., 2010).

While the outputs of our analyses indicate low levels of diseases risk, and higher levels of soil bacterial diversity being associated with mixed-animal farming, it is important to remember that soil bacterial communities are, by their very nature, highly variable, and are easily offset by a combination of both internal and external stimuli. Thus, these types of investigations can only benefit from longitudinal sampling efforts and surveillance, which would collectively improve the accuracy of analytical outputs and offer greater support to any deductions made from observed patterns within the data. Nevertheless, the outcomes of our study at the Krommelboog Farm, have highlighted the approach's ability to promote and maintain soil bacterial diversity, while simultaneously limiting the individual contributions of pathogens to the overall bacterial community composition. With that being said, the strategy implemented at the Krommelboog farm, by the Landmark Foundation, has demonstrated an ability to promote agricultural productivity, despite the limitations imposed by a lack of environmental resources.

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Appendix 1: User generated metadata mapping file, providing per sample metadata for a number of protocols within the QIIME 1.9.1 pipeline

#SampleID	BarcodeSequence	LinkerPrimerSequence	InputFastaName	SampleType	Location	Description
J.82	CCTAGAGTCTCTCTAC	AGATGTGTATAAGAGACAG	J82_merged.fasta	Dung	Beaufort	Livestock
J.83	GCGTAAGACTCTCTAC	AGATGTGTATAAGAGACAG	J83_merged.fasta	Dung	Beaufort	Contact
J.86	CTATTAAGCTCTCTAC	AGATGTGTATAAGAGACAG	J86_merged.fasta	Dung	Beaufort	Wildlife
J.87	AAGGCTATCTCTCTAC	AGATGTGTATAAGAGACAG	J87_merged.fasta	Soil	Beaufort	Wildlife
J.88	GAGCCTTACTCTCTAC	AGATGTGTATAAGAGACAG	J88_merged.fasta	Dung	Beaufort	Wildlife
J.89	TTATGCGACTCTCTAC	AGATGTGTATAAGAGACAG	J89_merged.fasta	Dung	Beaufort	Livestock
J.90	TCGACTAGCGAGGCTG	AGATGTGTATAAGAGACAG	J90_merged.fasta	Dung	Beaufort	Livestock
J.91	TTCTAGCTCGAGGCTG	AGATGTGTATAAGAGACAG	J91_merged.fasta	Dung	Beaufort	Contact
J.93	CCTAGAGTCGAGGCTG	AGATGTGTATAAGAGACAG	J93_merged.fasta	Dung	Beaufort	Wildlife
J.95	GCGTAAGACGAGGCTG	AGATGTGTATAAGAGACAG	J95_merged.fasta	Soil	Beaufort	Livestock
J.96	CTATTAAGCGAGGCTG	AGATGTGTATAAGAGACAG	J96_merged.fasta	Soil	Beaufort	Livestock
J.98	AAGGCTATCGAGGCTG	AGATGTGTATAAGAGACAG	J98_merged.fasta	Soil	Beaufort	Livestock

Appendix 2: List of bacterial phyla detected and classified from samples collected across “wildlife”, “livestock” and “contact” areas at the Krommelboog Farm, Beaufort West, South Africa

Kingdom	Phylum	Percentage
Bacteria	Firmicutes	28,20
Bacteria	Plantomycetes	19,70
Bacteria	Actinobacteria	12,50
Bacteria	TM7	9,00
Bacteria	Bacteroidetes	8,60
Archaea	Euryarchaeota	5,50
Bacteria	Verrucomicrobia	4,90
Bacteria	Chloroflexi	3,30
Bacteria	Proteobacteria	2,40
Bacteria	Acidobacteria	1,90
Bacteria	Cyanobacteria	0,90
Bacteria	OD1	0,40
Bacteria	Gemmatimonadetes	0,30
Bacteria	Synergistetes	0,20
Bacteria	Lentisphaerae	0,10
Archaea	Crenarchaeota	<0.01
Archaea	[Parvarchaeota]	<0.01
Bacteria	Armatimonadetes	<0.01
Bacteria	BHI80-139	<0.01
Bacteria	BRC1	<0.01
Bacteria	Chlorobi	<0.01
Bacteria	Elusimicrobia	<0.01
Bacteria	FBP	<0.01
Bacteria	Fibrobacteres	<0.01
Bacteria	Fusobacteria	<0.01
Bacteria	GN02	<0.01
Bacteria	Kazan-3B-28	<0.01
Bacteria	MVP-21	<0.01
Bacteria	Nitrospirae	<0.01
Bacteria	SR1	<0.01
Bacteria	Spirochaetes	<0.01
Bacteria	Tenericutes	<0.01
Bacteria	WPS-2	<0.01
Bacteria	Thermi	<0.01

Appendix 3: List of bacterial families detected and classified from samples collected across “wildlife”, “livestock” and “contact” areas at the Krommelboog Farm, Beaufort West, South Africa

Phylum	Class	Order	Family	Percentage
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	11,5
TM7	TM7-3	CW040	F16	8,1
Acidobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	6,8
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	5,7
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	5,5
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	4,7
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	4,6
Acidobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	4,4
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	1,9
Acidobacteria	Chloracidobacteria	RB41	Ellin6075	1,6
Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	1,5
Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	1,4
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	1,3
Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	1,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	0,9
Proteobacteria	Alphaproteobacteria	Sphingmonadales	Sphingomonadaceae	0,9
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	0,6
Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	0,6
Choloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	0,6
Choloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	0,6
Bacteroidetes	Bacteroidia	Bacteroidales	RF-16	0,4
Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	0,4
Bacteroidetes	Bacteroidia	Bacteroidales	Pophyromonadaceae	0,3
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	0,3
Firmicutes	Clostridia	Clostridiales	Mogibacteriaceae	0,3
Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	0,3
Acidobacteria	Actinobacteria	Actinomycetales	Geodermatophiliaceae	0,2
Acidobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	0,2
Bacteroidetes	Saprospirae	Saprospirales	Chtinophagaceae	0,2
Choloroflexi	Chloroflexi	Chloroflexales	Cholorflexaceae	0,2
Firmicutes	Clostridia	Clostridiales	Christensenellaceae	0,2
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	0,2
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	0,2
Proteobacteria	Alphaproteobacteria	Sphingmonadales	Erthrobacteraceae	0,2
Acidobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	0,1
Acidobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	0,1
Acidobacteria	Actinobacteria	Bifiobacteriales	Bifidobacteriaceae	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	0,1

Appendix 3: Continued

Phylum	Class	Order	Family	Percentage
Bacteroidetes	Bacteroidia	Bacteroidales	Odoribacteraceae	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	p-2534-18B5	0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	0,1
Firmicutes	Bacilli	Bacillales	Bacillaceae	0,1
Firmicutes	Bacilli	Bacillales	Planococcaceae	0,1
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	0,1
Firmicutes	Clostridia	Clostridiales	Veilonellaceae	0,1
Firmicutes	Erysipelotrichi	Eryipselotrichales	Erysipelotrichaceae	0,1
Lentisphaera	Lentisphaeria	Victivallales	Victivallaceae	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizoiaceae	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hypomicrobiaceae	0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	0,1
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterbacteriaceae	0,1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	0,1
Synergistetes	Synergistia	Synergistales	Dethiosulfovibroonaceae	0,1
Synergistetes	Synergistia	Synergistales	Synergistaceae	0,1
Acidobacteria	Acidobacteria-6	iii1-15	RB40	<0,1
Acidobacteria	Acidobacteria-7	iii1-15	mb2424	<0,1
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	<0,1
Acidobacteria	Solibacteres	Solibacteriales	PAUC26f	<0,1
Acidobacteria	Solibacteres	Solibacteriales	Solibacteraceae	<0,1
Acidobacteria	Acidimicrobiia	Acidomicrobiales	AKIw874	<0,1
Acidobacteria	Acidimicrobiia	Acidomicrobiales	C111	<0,1
Acidobacteria	Acidimicrobiia	Acidomicrobiales	EB1017	<0,1
Acidobacteria	Acidimicrobiia	Acidomicrobiales	Lamiaceae	<0,1
Acidobacteria	Acidimicrobiia	Acidomicrobiales	Microthrixaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Actinomycetaaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Actinosynnemataceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Bogoriellaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Corynebacteraceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Cryptosporangilaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Dietziaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Frankiaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Gordoniaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Jonesiaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	<0,1

Appendix 3: Continued

Phylum	Class	Order	Family	Percentage
Acidobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Proplonibacteriaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	<0,1
Acidobacteria	Actinobacteria	Actinomycetales	Sangulbacteraceae	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	BS11	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	SB-1	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Barnesiellaceae	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cyclobacteriaceae	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Flammeovigaceae	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	<0,1
Bacteroidetes	Sphingbacteriia	Sphingobacteriales	Sphingobacteriaceae	<0,1
Bacteroidetes	Rhodothermi	Rhodothermales	Rhodothermaceae	<0,1
Bacteroidetes	Saprospirae	Saprospirales	Saprospiraceae	<0,1
Chlorobi	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	<0,1
Choloroflexi	Chloroflexi	Chloroflexales	FFCH7168	<0,1
Choloroflexi	Chloroflexi	Chloroflexales	Oscillichloriaceae	<0,1
Choloroflexi	Chloroflexi	Roseflexales	Kouleothrixaceae	<0,1
Choloroflexi	Ktedonobacteria	Ktendobacterales	Ktendobacteraceae	<0,1
Choloroflexi	TK10	AKYG885	Dolo_23	<0,1
Choloroflexi	Thermobacula	Thermobaculales	Thermobaculaceae	<0,1
Choloroflexi	Anaerolineae	Ardenscatenales	Ardenscatenaceae	<0,1
Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	<0,1
Crenarchaeota	Thaumarchaeota	Nitrosphaerales	Nitrosphaeraceae	<0,1
Cyanobacteria	Nostocophycideae	Nostocales	Nostacaceae	<0,1
Cyanobacteria	Nostocophycideae	Stigonematales	Rivulariaceae	<0,1
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	<0,1
Cyanobacteria	Synechcoccophycideae	Pseudanabaenales	Pseudanabaeceae	<0,1
Cyanobacteria	Synechcoccophycideae	Synechococcales	Synechoccaceae	<0,1
Euryarchaeota	Methanobacteria	Methanomicrobiales	Methanoregulaceae	<0,1
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanospirillaceae	<0,1
Euryarchaeota	Methanobacteria	Methanosarcinales	Methanosaetaceae	<0,1
Euryarchaeota	Methanobacteria	Methanosarcinales	Methanosacinaceae	<0,1
Euryarchaeota	Thermoplasmata	E2	Methanomassiliicoccaceae	<0,1
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	<0,1
Fibrobacteres	TG3	TG3-1	TSCOR003-O20	<0,1
Firmicutes	Clostridia	Clostridiales	Dehalobacteriaceae	<0,1
Firmicutes	Clostridia	Clostridiales	EtOh8	<0,1

Appendix 3: Continued

Phylum	Class	Order	Family	Percentage
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	<0,1
Firmicutes	Clostridia	Clostridiales	Gracilibacteraceae	<0,1
Firmicutes	Clostridia	Clostridiales	Acidaminobacteraceae	<0,1
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	<0,1
Fusobacteria	Fusobacteria	Fusobacteria	Fusobacteriaceae	<0,1
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	A1-B1	<0,1
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Ellin5301	<0,1
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimondaceae	<0,1
Lentisphaera	Lentisphaeria	Z20	r4-45B	<0,1
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	<0,1
Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinkiaceae	<0,1
Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	<0,1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<0,1
Proteobacteria	Gammaproteobacteria	Xanthomondales	Sinobacteraceae	<0,1
Proteobacteria	Gammaproteobacteria	Xanthomondales	Xanthomondaceae	<0,1
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovirbrionaceae	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	Halianggiaceae	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	OM27	<0,1
Proteobacteria	Deltaproteobacteria	Entotheonellales	Entotheonellaceae	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Heliobacteraceae	<0,1
Proteobacteria	Gammaproteobacteria	Aeromondales	Aeromondaceae	<0,1
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Shewanellaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodobiaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyhomonadaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetbacteraceae	<0,1
Proteobacteria	Alphaproteobacteria	Ricketsiales	Mithochondria	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<0,1
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	<0,1
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	<0,1
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	<0,1
[Thermi]	Deinococci	Deinococcales	Trueperaceae	<0,1

Appendix 3: Continued

Phylum	Class	Order	Family	Percentage
Verrucomicrobia	Opitutae	Cerasiococcales	Cerasicoccaceae	<0,1
Verrucomicrobia	Pedosphaerae	Pedosphaerales	Ellin515	<0,1
Verrucomicrobia	Pedosphaerae	Pedosphaerales	Ellin517	<0,1
Verrucomicrobia	Spartobacteria	Chthoniobacterales	01D2Z36	<0,1

Appendix 4: List of bacterial genera detected and classified from soil and dung samples collected at the Krommelboog Farm, Beaufort West, South Africa

Phylum	Class	Order	Family	Genus	Percentage
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Arthrobacter	6,6
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia	4,6
Planctomyctes	Planctomycetia	Gemmatales	Gemmataceae	Gemmata	4,1
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanospaera	3,4
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Eggerthella	2,4
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	2
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	1,4
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio	0,9
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	0,9
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	0,9
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	0,8
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	0,8
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter	0,7
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	0,6
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Epulopiscium	0,6
Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	CF231	0,4
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	0,4
Planctomyctes	Planctomycetia	Pirellulales	Pirellulaceae	Pirellula	0,4
Planctomyctes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	0,4
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomondaceae	Kaistobacter	0,4
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella	0,3
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	Anaerolinea	0,3
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	SHD-231	0,3
Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	TRC22	0,2
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	5-7N15	0,2
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromondaceae	Paludibacter	0,2
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0,1
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Adlercreutzia	0,1
Bacteroidetes	Saprospirae	Saprospirales	Chitinophagaceae	Flavisolibacter	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Odobacteraceae	Buyricmonas	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	BF311	0,1
Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	Caldilinea	0,1
Chloroflexi	Chloroflexi	Chloroflexales	Cholorflexaceae	Cholronema	0,1
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0,1
Firmicutes	Bacilli	Lactobacillales	Lactoballilaceae	Lactobacillus	0,1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	0,1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Balneimonas	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella	0,1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomondaceae	Sphingomonas	0,1

Appendix 4: Continued

Phylum	Class	Order	Family	Genus	Percentage
Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	Candidatus Xiphinematobacter	0,1
Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	<0.1
Crenarchaeota	Thaumarchaeota	Nitrosphaerales	Nitrosphaeraceae	Candidatus Nitrososphaera	<0.1
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobacterium	<0.1
Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanolinea	<0.1
Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanospirillaceae	Methanospirillum	<0.1
Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae	Methanosaeta	<0.1
Euryarchaeota	Methanomicrobia	Methanosarcinales	Mathanosarcinaceae	Methanosarcina	<0.1
Euryarchaeota	Thermoplasmata	E2	Methanomassiliicoccaceae	Methanomassiliicoccus	<0.1
Euryarchaeota	Thermoplasmata	E2	Methanomassiliicoccaceae	vadinCA11	<0.1
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	<0.1
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	R18-435	<0.1
[Thermi]	Deinococci	Deinococcales	Trueperaceae	Truepera	<0.1
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	Candidatus Koribacterer	<0.1
Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	Candidatus Solibacter	<0.1
Actinobacteria	Acidimicrobiia	Acidomicrobiales	Lamiaceae	Lamia	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Bogoriellaceae	Georgenia	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Actinotalea	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Bracybacterium	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	Dietzia	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Geodermatophilus	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Modestobacter	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Gordoniaceae	Gordonia	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Janibacter	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Phycicoccus	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Terracoccus	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agromyces	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Salinibacterium	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Microbispora	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Nesterenkonia	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Actinoplanes	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Dactylosporangium	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Pilimelia	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Virdisporangium	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Myobacterium	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Aeromircobium	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Kribella	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Noocardioides	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Pimelobacter	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Actinomycetospora	<0.1

Appendix 4: Continued

Phylum	Class	Order	Family	Genus	Percentage
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudoonocardia	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Sangulbacteraceae	Sanguibacter	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Sporithyaceae	Sporicthya	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	<0.1
Actinobacteria	Actinobacteria	Actinomycetales	Thermomonospsoraceae	Actinomadura	<0.1
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	<0.1
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Slackia	<0.1
Actinobacteria	Nitriliruptoria	Euzebyales	Euzebyaceae	Euzebya	<0.1
Armatimonadetes	Fimbriimonadia	Fimbriiomondales	Rhodothermaceae	Fibriimonas	<0.1
Bacteroidetes	Rhodothermi	Rhodothermales	Chitinophagaceae	Rubricoccus	<0.1
Bacteroidetes	Saprospirae	Saprospirales	Chitinophagaceae	Chitinophaga	<0.1
Bacteroidetes	Saprospirae	Saprospirales	Chitinophagaceae	Flavihumibacter	<0.1
Bacteroidetes	Saprospirae	Saprospirales	Chitinophagaceae	Sediminibacterium	<0.1
Bacteroidetes	Saprospirae	Saprospirales	Chitinophagaceae	Segetibacter	<0.1
Bacteroidetes	Bacteroidia	Bacteroidales	Odobacteraceae	Odorbacter	<0.1
Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	Prevotella	<0.1
Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	Paraprevotella	<0.1
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromondaceae	Parabacteroides	<0.1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Adhaeribacter	<0.1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Larkinella	<0.1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Pontibacter	<0.1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Rhodocytophaga	<0.1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Runella	<0.1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Sporocytophaga	<0.1
Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	Fulvivirga	<0.1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	Chryseobacterium	<0.1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluvicola	<0.1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	<0.1
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	<0.1
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	<0.1
Chloroflexi	Thermobacula	Thermobaculales	Thermobaculaceae	Thermobaculum	<0.1
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	C1_B004	<0.1
Chloroflexi	Anaerolineae	Ardenscatenales	Ardenscatenaceae	Ardenscatena	<0.1
Chloroflexi	Chloroflexi	Roseiflexales	Kouleothrixaceae	Kouleothrix	<0.1
Chloroflexi	Chloroflexi	Chloroflexales	Oscilliochloridaceae	Oscillochloris	<0.1
Chloroflexi	Ktendobacteria	Ktendobacterales	Ktendobacteraceae	FFCH10602	<0.1
Cyanobacteria	Nostocophycideae	Nostocales	Nostocaceae	Nosctoc	<0.1
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	Phormidium	<0.1
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	Plantothrix	<0.1
Cyanobacteria	Synechococcophycideae	Pseudanabaenales	Pseudanabaenaceae	Leptolynbya	<0.1
Cyanobacteria	Synechococcophycideae	Synechococcales	Synechococcacaceae	Synechococcus	<0.1
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibriobacteraceae	Fibrobacter	<0.1
Firmicutes	Bacilli	Bacillales	Exiguobacteraceae	Exiguobacterium	<0.1

Appendix 4: Continued

Phylum	Class	Order	Family	Genus	Percentage
Firmicutes	Bacilli	Bacillales	Bacillaceae	Anaerobacillus	<0.1
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	<0.1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Kurthia	<0.1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinbacillus	<0.1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Rummelibacillus	<0.1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Solibacillus	<0.1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina	<0.1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Viridibacillus	<0.1
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Trichococcus	<0.1
Firmicutes	Bacilli	Lactobacillales	Enterobacteriaceae	Enterococcus	<0.1
Firmicutes	Bacilli	Turicibacterales	Turibacteraceae	Turibacter	<0.1
Firmicutes	Clostridia	Clostridiales	Acidaminobacteraceae	Fusibacter	<0.1
Firmicutes	Clostridia	Clostridiales	Mogibacteraceae	Anaerovorax	<0.1
Firmicutes	Clostridia	Clostridiales	Mogibacteraceae	Mogibacterium	<0.1
Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenalla	<0.1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Alkaphilus	<0.1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Caloramtor	<0.1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Proteiniclasticum	<0.1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	SMb53	<0.1
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Acetobacterium	<0.1
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Anaerofustis	<0.1
Firmicutes	Clostridia	Clostridiales	Gracilibacteraceae	Gracilibacter	<0.1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	<0.1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	<0.1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	<0.1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	<0.1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	<0.1
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Pelotomaculum	<0.1
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Clostridium	<0.1
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Tepidibacter	<0.1
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Anaerotruncus	<0.1
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	<0.1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Acidaminococcus	<0.1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Phascolarctobacterium	<0.1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Succiniclasticum	<0.1
Firmicutes	Eryspelotrichi	Erysipelotrichales	Erysipelotrichaceae	Bulleidia	<0.1
Firmicutes	Eryspelotrichi	Erysipelotrichales	Erysipelotrichaceae	Holdemania	<0.1
Firmicutes	Eryspelotrichi	Erysipelotrichales	Erysipelotrichaceae	RFN20	<0.1
Fusobacteria	Fusobacteriia	Fusobacterales	Fusobacteriaceae	Cetobacterium	<0.1
Fusobacteria	Fusobacteriia	Fusobacterales	Fusobacteriaceae	Fusobacterium	<0.1
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	<0.1
Nitrospirae	Nitrospira	Nitrosprales	Nitrospiraceae	Nitrospira	<0.1
Planctomyctes	Planctomycetia	Gemmatales	Isosphaeraceae	Nostocoida	<0.1

Appendix 4: Continued

Phylum	Class	Order	Family	Genus	Percentage
Planctomyctes	Planctomycetia	Pirellulales	Pirellulaceae	A17	<0.1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	<0.1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	<0.1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Mycoplana	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hypomicrobiaceae	Parvibaculum	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hypomicrobiaceae	Pedomicrobium	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hypomicrobiaceae	Rhodoplanes	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylosinus	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Pleomorphomonas	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobaceae	Agrobacterium	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodobaceae	Afifella	<0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Labrys	<0.1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomondaceae	Hyphomonas	<0.1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amaricoccus	<0.1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Anaerospora	<0.1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	<0.1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	<0.1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rubellimicrobium	<0.1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	<0.1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Magnetospirillum	<0.1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Phaeospirillum	<0.1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Rhodovibrio	<0.1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomondaceae	Novosphingobium	<0.1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomondaceae	Sphingobium	<0.1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomondaceae	Sphingopyxis	<0.1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonas	<0.1
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	<0.1
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	<0.1
Proteobacteria	Deltaproteobacteria	Entotheonellales	Entotheonellaceae	Candidatus Entheonella	<0.1
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	<0.1
Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	Anaeromyxobacter	<0.1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	<0.1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Heliobacteraceae	Sulfuricurvum	<0.1
Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	<0.1
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia	<0.1
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Plesiomonas	<0.1
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Serratia	<0.1
Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	Aquicella	<0.1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	<0.1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomondaceae	Pseudomonas	<0.1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Steroidobacter	<0.1

Appendix 4: Continued

Phylum	Class	Order	Family	Genus	Percentage
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	<0.1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	<0.1
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	<0.1
Synergistetes	Synergistia	Synergistales	Dethiosulfovibrionaceae	Pyramidobacter	<0.1
Synergistetes	Synergistia	Synergistales	Synergistaceae	Candidatus Tammella	<0.1
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	<0.1
Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	<0.1
Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	DA101	<0.1
Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	Ellin506	<0.1
Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	OR-59	<0.1
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteliobacter	<0.1
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prosthecobacter	<0.1

Appendix 5: User generated metadata mapping file, providing per sample metadata for a number of protocols within the QIIME 1.9.1 pipeline

#SampleID	BarcodeSequence	LinkerPrimerSequence	InputFastaName	SampleType	Location	Description
J.82	CCTAGAGTCTCTCTAC	AGATGTGTATAAGAGACAG	J82_merged.fasta	Dung	Beaufort	Segregated
J.86	CTATTAAGCTCTCTAC	AGATGTGTATAAGAGACAG	J86_merged.fasta	Dung	Beaufort	Segregated
J.87	AAGGCTATCTCTCTAC	AGATGTGTATAAGAGACAG	J87_merged.fasta	Soil	Beaufort	Segregated
J.88	GAGCCTTACTCTCTAC	AGATGTGTATAAGAGACAG	J88_merged.fasta	Dung	Beaufort	Segregated
J.89	TTATGCGACTCTCTAC	AGATGTGTATAAGAGACAG	J89_merged.fasta	Dung	Beaufort	Segregated
J.90	TCGACTAGCGAGGCTG	AGATGTGTATAAGAGACAG	J90_merged.fasta	Dung	Beaufort	Segregated
J.93	CCTAGAGTCGAGGCTG	AGATGTGTATAAGAGACAG	J93_merged.fasta	Dung	Beaufort	Segregated
J.95	GCGTAAGACGAGGCTG	AGATGTGTATAAGAGACAG	J95_merged.fasta	Soil	Beaufort	Segregated
J.96	CTATTAAGCGAGGCTG	AGATGTGTATAAGAGACAG	J96_merged.fasta	Soil	Beaufort	Segregated
J.98	AAGGCTATCGAGGCTG	AGATGTGTATAAGAGACAG	J98_merged.fasta	Soil	Beaufort	Segregated
YB.1	CATACACTGT	AGATGTGTATAAGAGACAG	YB1_merged.fasta	Dung	Beaufort	Integrated
YB.2	GTGTGGCGCT	AGATGTGTATAAGAGACAG	YB2_merged.fasta	Dung	Beaufort	Integrated
YB.3	ATCACGAAGG	AGATGTGTATAAGAGACAG	YB3_merged.fasta	Soil	Beaufort	Integrated
YB.4	CGGCTCTACT	AGATGTGTATAAGAGACAG	YB4_merged.fasta	Dung	Beaufort	Integrated
YB.5	GAATGCACGA	AGATGTGTATAAGAGACAG	YB5_merged.fasta	Soil	Beaufort	Integrated

Appendix 5: Continued

YB.6	AAGACTATAG	AGATGTGTATAAGAGACAG	YB6_merged.fasta	Soil	Beaufort	Integrated
YB.7	TCGGCAGCAA	AGATGTGTATAAGAGACAG	YB7_merged.fasta	Dung	Beaufort	Integrated
YB.8	CTAATGATGG	AGATGTGTATAAGAGACAG	YB8_merged.fasta	Soil	Beaufort	Integrated
YB.9	GGTTGCCTCT	AGATGTGTATAAGAGACAG	YB9_merged.fasta	Soil	Beaufort	Integrated
YB.10	CGCACATGGC	AGATGTGTATAAGAGACAG	YB10_merged.fasta	Dung	Beaufort	Integrated
YB.11	GGCCTGTCCT	AGATGTGTATAAGAGACAG	YB11_merged.fasta	Soil	Beaufort	Integrated
YB.12	CTGTGTTAGG	AGATGTGTATAAGAGACAG	YB12_merged.fasta	Soil	Beaufort	Integrated
YB.13	TAAGGAACGT	AGATGTGTATAAGAGACAG	YB13_merged.fasta	Soil	Beaufort	Integrated
YB.14	CTAACTGTAA	AGATGTGTATAAGAGACAG	YB14_merged.fasta	Dung	Beaufort	Integrated
YB.15	GGCGAGATGG	AGATGTGTATAAGAGACAG	YB15_merged.fasta	Soil	Beaufort	Integrated
YB.16	AATAGAGCAA	AGATGTGTATAAGAGACAG	YB16_merged.fasta	Dung	Beaufort	Integrated

Appendix 6: List of bacterial phyla detected and classified from samples collected across “integrated” and “segregated” areas at the Krommelboog Farm, Beaufort West, South Africa.

Kingdom	Phylum	Percentage
Bacteria	Planctomycetes	30,8
Bacteria	Firmicutes	13,4
Bacteria	Actinobacteria	13,1
Bacteria	Bacteroidetes	8,5
Bacteria	Proteobacteria	7,1
Bacteria	Verrucomicrobia	6,5
Bacteria	Acidobacteria	3,9
Bacteria	Chloroflexi	3,7
Archaea	Euryarchaeota	3,2
Bacteria	TM7	3,2
Bacteria	Cyanobacteria	2,9
Bacteria	Gemmatimonadetes	0,8
Bacteria	[Thermi]	0,3
Bacteria	OD1	0,2
Bacteria	WPS-2	0,2
Bacteria	Armatimonadetes	0,1
Bacteria	Lentisphaerae	0,1
Bacteria	WS4	0,1
Archaea	Crenarchaeota	<0,1
Archaea	[Parvarchaeota]	<0,1
Bacteria	AD3	<0,1
Bacteria	BRC1	<0,1
Bacteria	Chlorobi	<0,1
Bacteria	Elusimicrobia	<0,1
Bacteria	FBP	<0,1
Bacteria	Fibrobacteres	<0,1
Bacteria	Fusobacteria	<0,1
Bacteria	GAL15	<0,1
Bacteria	GN02	<0,1
Bacteria	MVP-21	<0,1
Bacteria	NKB19	<0,1
Bacteria	Nitrospirae	<0,1
Bacteria	SR1	<0,1
Bacteria	Spirochaetes	<0,1
Bacteria	Synergistetes	<0,1
Bacteria	Tenericutes	<0,1
Bacteria	WS2	<0,1
Bacteria	WS5	<0,1

Appendix 7: List of bacterial families detected and classified from samples collected across "integrated" and "segregated" areas at the Krommelboog Farm, Beaufort West, South Africa

Phylum	Class	Order	Family	Percentage
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	8,8
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	5,9
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	5,4
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	4,2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	3,3
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	3,2
Acidobacteria	[Chloroacidobacteria]	RB41	Ellin6075	3,1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	2,5
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	2,4
Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	2,3
TM7	TM7	CW040	F16	2,1
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	1,4
Bacteroidetes	Bacteroidia	Bacteroidales	[Parapevotellaceae]	1,3
Cyanobacteria	Nostocophycideae	Nostocales	Nostacaceae	1,0
Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	1,0
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	0,9
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophillaceae	0,8
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	0,8
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	0,6
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	0,6
Verrucomicrobia	[Spartobacteria]	[Chthoniobacteriales]	[Chthoniobacteriaceae]	0,6
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	0,5
Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	0,5
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteriaceae	0,5
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	0,5
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	0,4
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	0,4
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	0,4
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	0,4
Chloroflexi	Chloroflexi	Chloroflexales	Cholorflexaceae	0,4
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	0,4
Acidobacteria	Acidobacteriia	Acidobacteriales	Koriobacteraceae	0,3
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobactereaceae	0,3
Bacteroidetes	Bacteroidia	Bacteroidales	RF16	0,3
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	0,3
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	0,3
Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	0,3
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	0,3
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	0,3

Appendix 7: Continued

Phylum	Class	Order	Family	Percentage
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	0,3
Bacteroidetes	Bacteroidia	Bacteroidales	ML635J-40	0,2
Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	0,2
Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	0,2
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidaceae	0,2
Cyanobacteria	Synechococcophycideae	Pseudoanabaenales	Pseudoanabaenaceae	0,2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijierinckiaceae	0,2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	0,2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	0,2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	0,2
[Thermi]	Deinococci	Deinococcales	Trueperaceae	0,2
Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Sporichthyaceae	0,1
Armatimonadetes	[Fimbriimonadia]	[Fibriimonadales]	[Fibriimonadaceae]	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	BA008	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	SB-1	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	p-2534-18B5	0,1
Bacteroidetes	Cytophagia	Cytophagales	Cyclobacteriaceae	0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	0,1
Firmicutes	Clostridia	Clostridiales	Christensenellaceae	0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	0,1
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	0,1
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Ellin5301	0,1
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	0,1
Lentisphaerae	[Lentisphaeria]	Victivallales	Victivallaceae	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	0,1
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	0,1
Crenarchaeota	Thaumarchaeota	Nitrosphaerales	Nitrosphaeraceae	<0,1
Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	<0,1
Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	<0,1
Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae	<0,1
Euryarchaeota	Methanomicrobia	Methanosarcinales	Methansarcinaceae	<0,1
Acidobacteria	Acidobacteria-6	Iii1-15	mb2424	<0,1
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	<0,1
Acidobacteria	Solibacteres	Solibacterales	AKIW659	<0,1
Acidobacteria	Solibacteres	Solibacterales	PAUC26f	<0,1
Acidobacteria	Solibacteres	Solibacterales	[Bryobacteraceae]	<0,1

Appendix 7: Continued

Phylum	Class	Order	Family	Percentage
Actinobacteria	Acidomicrobiia	Acidimicrobiales	AKIW874	<0,1
Actinobacteria	Acidomicrobiia	Acidimicrobiales	C111	<0,1
Actinobacteria	Acidomicrobiia	Acidimicrobiales	EB1017	<0,1
Actinobacteria	Acidomicrobiia	Acidimicrobiales	Lamiaceae	<0,1
Actinobacteria	Acidomicrobiia	Acidimicrobiales	JdFBGBact	<0,1
Actinobacteria	Acidomicrobiia	Acidimicrobiales	Microthrixaceae	<0,1
Actinobacteria	Acidomicrobiia	Acidimicrobiales	koli13	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Actinosynnemataceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Bogoriellaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Cryptosporangiaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Franklaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Streptomyceteaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	<0,1
Actinobacteria	Nitriliruptoria	Euzebyales	Euzybyaceae	<0,1
Actinobacteria	Nitrilruptoria	Nitriliruptorales	Nitriliruptoraceae	<0,1
Actinobacteria	Thermoleophilia	Gaiellales	AK1AB1_02E	<0,1
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	<0,1
Armatimonadetes	Armatimonadetes	Armatimonadales	Armatimonadaceae	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	BS11	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	[Barnesiellaceae]	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	<0,1
Bacteroidetes	Cytophagia	Cytophagales	[Amoebophilaceae]	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	<0,1
Bacteroidetes	[Rhodothermi]	[Rhodothermales]	Rhodothermaceae	<0,1
Bacteroidetes	Saprospirae	[Saprosirales]	Saprospiraceae	<0,1
Chlorobi	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	<0,1
Chloroflexi	Anaerolineae	A31	S47	<0,1
Chloroflexi	Anaerolineae	Ardenscatenales	Ardenscatenaceae	<0,1

Appendix 7: Continued

Phylum	Class	Order	Family	Percentage
Chloroflexi	Anaerolineae	SBR1031	A4b	<0,1
Chloroflexi	Anaerolineae	SBR1031	SJA-101	<0,1
Chloroflexi	Anaerolineae	SBR1031	oc28	<0,1
Chloroflexi	Chloroflexi	Chloroflexales	FFCH7168	<0,1
Chloroflexi	Chloroflexi	Chloroflexales	Oscillochloridaceae	<0,1
Chloroflexi	Dehalococcoidetes	Dehalococcoidales	Dehalococcoidaceae	<0,1
Chloroflexi	Ktedonobacteria	Ktedonobacteriales	Ktedonobacteriaceae	<0,1
Chloroflexi	TK10	AKYG885	5B-12	<0,1
Chloroflexi	TK10	AKYG885	Dolo-23	<0,1
Chloroflexi	TK10	B07_WMSP1	FFCH4570	<0,1
Chloroflexi	[Thermobacula]	[Thermobaculales]	[Thermobaculaceae]	<0,1
Cyanobacteria	Chloroplast	Chlorophyta	Chlamydomonodaceae	<0,1
Cyanobacteria	Chloroplast	Chlorophyta	Trebouxiophyceae	<0,1
Cyanobacteria	Nostocophycideae	Nostocales	Scytonemataceae	<0,1
Cyanobacteria	Nostocophycideae	Stigonematales	Rivulariaceae	<0,1
Cyanobacteria	Oscillatoriophycideae	Chroococcales	Xenococcaceae	<0,1
Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	<0,1
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobactereaceae	<0,1
Fibrobacteres	TG3	TG3-1	TSCOR003-O20	<0,1
Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	<0,1
Firmicutes	Bacilli	Bacillales	Bacillaceae	<0,1
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	<0,1
Firmicutes	Bacilli	Bacillales	Planococcaceae	<0,1
Firmicutes	Bacilli	Turicibacterales	Turibacteraceae	<0,1
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	<0,1
Firmicutes	Clostridia	Clostridiales	Gracilibacteraceae	<0,1
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	<0,1
Firmicutes	Clostridia	Clostridiales	[Acidaminobacteraceae]	<0,1
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	<0,1
Firmicutes	Clostridia	Natranaerobiales	Anaerobrancaceae	<0,1
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	<0,1
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	<0,1
Gemmmatimonadetes	Gemmatimonadetes	Gemmatimonadales	A1-B1	<0,1
Lentisphaerae	[Lenitsphaeria]	Z20	R4-45B	<0,1
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodobiaceae	<0,1

Appendix 7: Continued

Phylum	Class	Order	Family	Percentage
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	<0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	<0,1
Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<0,1
Proetobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	<0,1
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	<0,1
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	OM27	<0,1
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteriaceae	<0,1
Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacteraceae	Heliobacteraceae	<0,1
Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	<0,1
Proteobacteria	Gammaproteobacteria	Alteromonadales	211ds20	<0,1
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	<0,1
Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	<0,1
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	<0,1
Proteobacteria	Gammaproteobacteria	Pseduomonadales	Moraxellaceae	<0,1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<0,1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<0,1
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	<0,1
Synergistetes	Synergistia	Synergistales	Dethiosulfovibrionaceae	<0,1
Synergistetes	Synergistia	Synergistales	Synergistaceae	<0,1
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	<0,1
Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	<0,1
Verrucomicrobia	Opitutae	[Cerasicoccales]	[Cerasicoccaceae]	<0,1
[Thermi]	Deinococci	Thermales	Thermaceae	<0,1

Appendix 8: List of bacterial genera detected and classified from samples collected across “integrated” and “segregated” areas at the Krommelboog Farm, Beaufort West, South Africa

Phylum	Class	Order	Family	Genus	Percentage
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter	8,5
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia	5,8
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	Gemmata	4
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	2,3
Unassigned	Unassigned	Unassigned	Unassigned	Unassigned	1,6
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	1,4
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	1,2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Kaistobacter	1,2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	1,1
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	0,9
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Epulopiscium	0,8
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter	0,7
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0,6
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio	0,4
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	0,4
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	DA101	0,4
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Actinotalea	0,3
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0,3
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter	0,3
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	Anaerolinea	0,3
Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexaceae	Chloronema	0,3
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	0,3
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	0,3
Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	Pirellula	0,3
Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	0,3
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	0,3
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	Candidatus Koribacter	0,2
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Modestobacter	0,2
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Microbispora	0,2
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Aeromicrobium	0,2
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella	0,2
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	CF231	0,2
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Flavisolibacter	0,2
Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	Kouleothrix	0,2
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	Phormidium	0,2
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Caloramator	0,2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	0,2
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	0,2
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella	0,2
Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	Candidatus Solibacter	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas	0,1

Appendix 8: Continued

Phylum	Class	Order	Family	Genus	Percentage
Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	Dietzia	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Geodermatophilus	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Actinoplanes	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia	0,1
Armatimonadetes	[Fimbriimonadia]	[Fimbriimonadales]	[Fimbriimonadaceae]	Fimbriimonas	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	YRC22	0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Pontibacter	0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	0,1
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0,1
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	SHD-231	0,1
Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	Caldilinea	0,1
Cyanobacteria	Synechococcophycideae	Pseudanabaenales	Pseudanabaenaceae	Leptolyngbya	0,1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Alkaliphilus	0,1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Proteiniclasticum	0,1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	0,1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	0,1
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	0,1
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	0,1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Balneimonas	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Pleomorphomonas	0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rubellimicrobium	0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas	0,1
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	C. Xiphinematobacter	0,1
Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	<0,1
Crenarchaeota	Thaumarchaeota	Nitrososphaerales	Nitrososphaeraceae	C. Nitrososphaera	<0,1
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobacterium	<0,1
Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanolinea	<0,1
Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae	Methanosaeta	<0,1
Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanolobus	<0,1
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Edaphobacter	<0,1
Actinobacteria	Acidimicrobiia	Acidimicrobiales	Iamiaceae	Iamia	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Bogoriellaceae	Georgenia	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	Brevibacterium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Brachybacterium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	Kineococcus	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agromyces	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Clavibacter	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Microbacterium	<0,1

Appendix 8: Continued

Phylum	Class	Order	Family	Genus	Percentage
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Salinibacterium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Dactylosporangium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Pilimelia	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Virgisporangium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Friedmanniella	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Kribbella	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Pimelobacter	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Propionicimonas	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae	Xylanimicrobium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Actinomycetospora	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Amycolatopsis	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Saccharomonospora	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	Nonomuraea	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	Sphaerisporangium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	Streptosporangium	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	Actinocorallia	<0,1
Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	Actinomadura	<0,1
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Adlercreutzia	<0,1
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	<0,1
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Eggerthella	<0,1
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Slackia	<0,1
Actinobacteria	Nitriliruptoria	Euzebyales	Euzebyaceae	Euzebya	<0,1
Actinobacteria	Nitriliruptoria	Nitriliruptorales	Nitriliruptoraceae	Nitriliruptor	<0,1
Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae	Armatimonas	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	5-7N15	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	<0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Blvii28	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Adhaeribacter	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Dyadobacter	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Flectobacillus	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Leadbetterella	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Rhodocytophaga	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Rudanella	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Runella	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Spirosoma	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Sporocytophaga	<0,1
Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	Fulvivirga	<0,1
Bacteroidetes	Cytophagia	Cytophagales	[Amoebophilaceae]	C. Amoebophilus	<0,1

Appendix 8: Continued

Phylum	Class	Order	Family	Genus	Percentage
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Crocinitomix	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Gillisia	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Cloacibacterium	<0,1
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	<0,1
Bacteroidetes	[Rhodothermi]	[Rhodothermales]	Rhodothermaceae	Rubricoccus	<0,1
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Chitinophaga	<0,1
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	<0,1
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Segetibacter	<0,1
Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	Haliscomenobacter	<0,1
Chloroflexi	Anaerolineae	Ardenscatenales	Ardenscatenaceae	Ardenscatena	<0,1
Chloroflexi	Chloroflexi	Chloroflexales	Oscillochloridaceae	Oscillochloris	<0,1
Chloroflexi	[Thermobacula]	[Thermobaculales]	[Thermobaculaceae]	Thermobaculum	<0,1
Cyanobacteria	Nostocophycideae	Nostocales	Nostocaceae	Nodularia	<0,1
Cyanobacteria	Nostocophycideae	Nostocales	Nostocaceae	Nostoc	<0,1
Cyanobacteria	Nostocophycideae	Stigonematales	Rivulariaceae	Calothrix	<0,1
Cyanobacteria	Oscillatoriophycideae	Chroococcales	Xenococcaceae	Chroococcidiopsis	<0,1
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	Oscillatoria	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Crocinitomix	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Gillisia	<0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Cloacibacterium	<0,1
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	<0,1
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	Planktothrix	<0,1
Cyanobacteria	Synechococcophycideae	Pseudanabaenales	Pseudanabaenaceae	Pseudanabaena	<0,1
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	<0,1
Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	Alicyclobacillus	<0,1
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	<0,1
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	<0,1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	<0,1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Solibacillus	<0,1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina	<0,1
Firmicutes	Bacilli	Turicibacterales	Turicibacteraceae	Turicibacter	<0,1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Geosporobacter_Thermotalea	<0,1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Natronincola_Anaerovirgula	<0,1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	SMB53	<0,1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Sarcina	<0,1
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Tindallia_Anoxynatronum	<0,1
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Anaerofustis	<0,1
Firmicutes	Clostridia	Clostridiales	Gracilibacteraceae	Gracilibacter	<0,1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	<0,1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	<0,1

Appendix 8: Continued

Phylum	Class	Order	Family	Genus	Percentage
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	<0,1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	<0,1
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Desulfotomaculum	<0,1
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Pelotomaculum	<0,1
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	<0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Megasphaera	<0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Pelosinus	<0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Phascolarctobacterium	<0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Sporomusa	<0,1
Firmicutes	Clostridia	Clostridiales	[Acidaminobacteraceae]	Fusibacter	<0,1
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Anaerovorax	<0,1
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Mogibacterium	<0,1
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Sedimentibacter	<0,1
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Bulleidia	<0,1
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Cetobacterium	<0,1
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	<0,1
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	<0,1
Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	A17	<0,1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	<0,1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	<0,1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Mycoplana	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Pedomicrobium	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Phyllobacterium	<0,1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	<0,1
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	<0,1
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Desulfotomaculum	<0,1
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Pelotomaculum	<0,1
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	<0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Megasphaera	<0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Pelosinus	<0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Phascolarctobacterium	<0,1
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Sporomusa	<0,1
Firmicutes	Clostridia	Clostridiales	[Acidaminobacteraceae]	Fusibacter	<0,1
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Anaerovorax	<0,1
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Mogibacterium	<0,1
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Sedimentibacter	<0,1
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Bulleidia	<0,1
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Cetobacterium	<0,1
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	<0,1
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	<0,1

Appendix 8: Continued

Phylum	Class	Order	Family	Genus	Percentage
Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	A17	<0,1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	<0,1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	<0,1
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Mycoplana	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Pedomicrobium	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Phyllobacterium	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Kaistia	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodobiaceae	Afifella	<0,1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Labrys	<0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Hyphomonas	<0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amaricoccus	<0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Anaerospora	<0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Loktanella	<0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	<0,1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobaca	<0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseococcus	<0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	<0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Inquilinus	<0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Novispirillum	<0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Phaeospirillum	<0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Rhodovibrio	<0,1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Telmatospirillum	<0,1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Lutibacterium	<0,1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	<0,1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Pigmentiphaga	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonas	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Hydrogenophaga	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Methylibium	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Paucibacter	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Ramlibacter	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax	<0,1
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	<0,1
Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	Methylotenera	<0,1
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Dechloromonas	<0,1
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Propionivibrio	<0,1
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Uliginosibacterium	<0,1
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Zoogloea	<0,1
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	<0,1

Appendix 8: Continued

Phylum	Class	Order	Family	Genus	Percentage
Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	Nannocystis	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	Plesiocystis	<0,1
Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	C.Entotheonella	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Sulfurospirillum	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Flexispira	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Sulfuricurvum	<0,1
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Cellvibrio	<0,1
Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	<0,1
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	<0,1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	<0,1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	<0,1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas	<0,1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	<0,1
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	<0,1
Synergistetes	Synergistia	Synergistales	Dethiosulfovibrionaceae	Pyramidobacter	<0,1
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	<0,1
Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	<0,1
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	<0,1
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prosthecobacter	<0,1
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Chthoniobacter	<0,1
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Ellin506	<0,1
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	OR-59	<0,1
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	<0,1
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	R18-435	<0,1
[Thermi]	Deinococci	Deinococcales	Trueperaceae	B-42	<0,1
[Thermi]	Deinococci	Deinococcales	Trueperaceae	Truepera	<0,1
[Thermi]	Deinococci	Thermales	Thermaceae	Meiothermus	<0,1
[Thermi]	Deinococci	Thermales	Thermaceae	Thermus	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	Nannocystis	<0,1
Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	Plesiocystis	<0,1
Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	C. Entotheonella	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Sulfurospirillum	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Flexispira	<0,1
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Sulfuricurvum	<0,1
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Cellvibrio	<0,1
Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	<0,1
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	<0,1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	<0,1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	<0,1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas	<0,1