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Impacts of long-term plant residue management on soil organic matter quality, *Pseudomonas* community structure and disease suppressiveness

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ABSTRACT

The microbiome of grassland soils provides ecosystem services essential to plant health and productivity, including nutrient cycling and suppression of soil-borne diseases. Understanding how soil management practices affect soil microbial communities will provide opportunities by which indigenous soil microbes and their functions can be managed to sustain or promote plant growth and enhance disease suppressiveness. Here, we investigated the impact of 20 years of plant residue management in a long-term grassland field trial on soil chemical and (micro)biological properties, in particular the suppression of damping-off disease of kale caused by the fungal root pathogen *Rhizoctonia solani* AG 2–1. Plant residue management led to significant variation in the community structure of the bacterial genus *Pseudomonas* between treatments. Soil organic matter quality (inferred carbon recalcitrance) was responsible for 80% of the observed variation in *Pseudomonas* community structure. Furthermore, increased *Pseudomonas* species diversity (Shannon's index), microbial activity, soil organic matter content, and carbon availability distinguished suppressive (low disease) soils from conducive (high disease) soils. More specifically, *Pseudomonas* species diversity and richness (Margalef's) were identified as the primary parameters explaining the greatest proportion (> 30%) of variation in the disease suppressive capacity of soils across treatments. Collectively, our results suggest that management-induced shifts in *Pseudomonas* community composition, notably species diversity and richness, provide a better indicator of disease conduciveness for a broad-host range fungal pathogen than soil chemical parameters. In conclusion, our study indicates that frequent addition of organic residues to agricultural grassland soils enhances the diversity and activity of plant-beneficial bacterial taxa.

1. Introduction

Plant health and productivity are reliant on the ecosystem services provided by indigenous soil and plant-associated microbiomes. These ecosystem services include organic matter storage and decomposition, nutrient cycling, and the suppression of soil-borne diseases (Janvier et al., 2007). Soil-borne plant pathogens are responsible for considerable yield losses in both arable (Raaijmakers et al., 2009) and pastoral agriculture (Dignam et al., 2016), yet understanding how and to what extent soil physical, chemical and biological properties affect disease onset and development remains a complex challenge. Pastoral-based agriculture, supporting the production of grazing livestock, covers over 25% of the Earth's ice-free land surface (FAOSTAT, 2011). In these systems, plant disease control is further complicated by the multi-plant multi-pathogen complexes that develop with perennial plant species, and the expansive nature and potentially challenging topography of pastoral farming systems that hinder practicable delivery of external inputs (Dignam et al., 2016).

Naturally disease suppressive soils are those in which consortia of indigenous microbes protect susceptible plant hosts from soil-borne disease, despite the presence of a virulent pathogen (Mendes et al., 2011; Penton et al., 2014; Cha et al., 2015; Raaijmakers and Mazzola, 2016). This phenomenon is typically divided into general suppression, driven by the competitive activity of the total soil microbiota, and specific suppression, driven by the antagonistic potential of an individual or specific consortia of microorganisms (Weller et al., 2002;

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Raaijmakers and Mazzola, 2016). The wealth of understanding gained from well-characterised examples of soil suppressiveness in arable systems (Weller et al., 2002; Cha et al., 2015; van der Voort et al., 2016; Carrión et al., 2018; Siegel-Hertz et al., 2018), and increasing understanding of the processes that underlie release from pathogen pressure in natural grassland systems (Maron et al., 2011; Schnitzer et al., 2011; Latz et al., 2012, 2016; Mommer et al., 2018), provide opportunities to explore and exploit such mechanisms in pastoral systems. Yet, little is known about the distribution of disease-suppressive communities in agricultural grasslands, nor how these communities and their functions relate or respond to soil management practices (Dignam et al., 2016; Wakelin, 2018). Understanding the soil physicochemical properties impacting upon microbial communities and their activities will enable the design of management practices to steer these ecosystem services (Nielsen et al., 2015).

A phylogenetically diverse range of soil microorganisms have been linked with plant disease suppression (Raaijmakers and Mazzola, 2012). Among these, *Pseudomonas* spp. have been repeatedly implicated and have been shown to be responsive to varying management practices across agricultural systems (Sarniguet et al., 1992; Garbeva et al., 2004; van Overbeek et al., 2012; Walters et al., 2018). In a recent study, we conducted an in-depth molecular analysis across pastoral soils to identify opportunities by which indigenous soil microbes may be managed to enhance soil suppressiveness (Dignam et al., 2018). Notably, changes in soil organic matter (SOM) quality were associated with both taxonomic (*Pseudomonas* community composition) and functional indicators of the disease suppressive potential in these soils. Therefore, management practices that result in alteration of SOM content and quality (e.g. chemical composition and decomposability), may provide opportunities to enhance soil suppressiveness. Research areas of direct importance to pastoral systems include incorporation of crop residues and animal stocking rates (i.e. impacts of grazing intensity and conversion of plant biomass to excreta) and plant species selection (i.e. crop-specific exudation of labile C into the rhizosphere).

This study assessed the impacts of varied plant residue management on soil chemical, physical and microbiological parameters in a long-term, grassland field trial. The contributions of management-induced changes in biotic and abiotic soil properties to soil suppressiveness were investigated. At the site selected for this study, previous measures of soil organic matter quantity and quality were shown to differ with plant residue management treatments (Simpson et al., 2012; Adair et al., 2013). The treatments associated with this site provided a unique experimental platform to test our research hypotheses under long-term, field-based conditions. Phylogenetic community analysis using the Illumina MiSeq platform targeted *Pseudomonas* spp., and a bioassay for disease suppressiveness was applied to the pasture soils utilising a broad-range plant pathogen (*R. solani* AG2-1) and non-pasture host (kale; *Brassica oleracea*). Measuring disease incidence on host plants unrelated to the pasture system's current crop species is likely to provide a better comparative measure of general, rather than specific, suppression between the field treatments (Dignam et al., 2015).

We hypothesised that: i) frequent additions of plant litter will lead to increased organic matter content (SOM quantity) and labile C (SOM quality); ii) *Pseudomonas* species composition will respond to changes in SOM quality, resulting in increased *Pseudomonas* species diversity with increased supplies of labile C; and iii) through management-induced changes in *Pseudomonas* community structure, increased *Pseudomonas* diversity will lead to enhanced soil suppressiveness.

2. Materials and methods

2.1. Long-term grassland trial: field site and mowing treatments

A long-term ecology field trial with varied fertiliser application and plant residue management was established in 1994 at Lincoln University (Lincoln, New Zealand; Simpson et al., 2012). The trial was established on a Wakanui silt loam soil (mottled immature Pallic (NZ); Udic Ustochrept (USDA); Simpson et al., 2012). The trial comprised of four replicate plots (25 m^2) per treatment, arranged in a randomised block design. Each plot was planted to a mixture of red clover (*Trifolium pratense*), white clover (*Trifolium repens*), perennial ryegrass (*Lolium perenne*), and cocksfoot (*Dactylis glomerata*). The trial was not grazed or irrigated, and the treatments selected for analysis had not received mineral fertiliser. The treatments selected for this study combined two factors: mowing frequency and mown biomass removal. The four treatments were: 'FL' – mown frequently (when sward height reaches 15 cm; 7 to 9 times per annum) and biomass left; 'IL' – mown infrequently (when sward height reaches 30 cm; 5 to 7 times per annum) and biomass left; 'IR' – mown infrequently and biomass removed; 'NM' – never mown.

Botanical composition for each of the treatments was documented in 2011, 17 years after the field trial had been established (pers. comm. Hannah Buckley, Lincoln University; Adair et al., 2013). The dominant plant species for each treatment (those present in at least 3 of 5 quadrats for at least 3 of 4 replicate plots) were: white clover, perennial ryegrass, yarrow (*Achillea millefolium*) and chickweed (*Stellaria media*) (FL); white clover, perennial ryegrass, cocksfoot, yarrow and chickweed (IL); white clover, perennial ryegrass, cocksfoot and yarrow (IR); and Cocksfoot (NM).

2.2. Soil sampling and chemistry

Soil sampling was carried out in November 2014 when the field trial had been running for 20 years. Soil was sampled to a depth of 12.5 cm using soil corers with 2.5 cm diameter. Sixty soil cores (approx. 1.5 kg soil per plot) were collected spanning the length and width of each plot. Soil samples from each plot were pooled and sieved to 2 mm to remove plant debris and stones, and stored at 4 °C until use.

Physicochemical properties of a composite soil sample from each field plot were characterised by RJ Hill Laboratories (Christchurch, New Zealand). The following properties were measured: pH(water); Olsen P; total C; total N; total P; organic matter (%); anaerobically mineralisable nitrogen (AMN); available nitrogen (AN); K; Ca; Mg; Na; Al; phosphate retention; cation exchange capacity (CEC); total base saturation; and volume weight (http://www.hill-laboratories.com/file/fileid/15530). Ca, K, Mg and Na were expressed as me/100 g. C:N, C:P and AMN:N ratios were calculated from total C, N, P and AMN measurements. Hot water extractable C (HWEC) and dissolved organic C (DOC) aromatic content were quantified as described in Dignam et al. (2018). Briefly, HWEC was extracted from soil (Ghani et al., 2003), and solutions analysed using a Shimadzu 5000A TOC analyser. HWEC solutions were normalized by total dissolved organic carbon (DOC = total carbon – inorganic carbon) to 45 μg/ml, and the aromatic component of the carbon (DOC aromatic content) quantified by UV absorbance at 254 nm (FLUOstar Omega microplate reader, BMG Labtech (Volk et al., 2002). DOC aromatic content (DOC AC) provides a measure of carbon recalcitrance and availability to the microbial community; we infer that higher DOC AC is indicative of greater carbon recalcitrance and reduced availability. Soil moisture content was measured by incubating subsamples (20 g) of each soil at 80 °C until no change in sample weight was detected over 2 consecutive days.

2.3. DNA extraction

DNA was extracted from the composite soil of each field plot using the PowerSoil DNA extraction kit (MoBio Inc, USA), according to the manufacturer's instructions. Duplicate extractions were made from 0.25 g of soil and the extracts combined to provide 100 μl of DNA which was stored at −20 °C. DNA was quantified by spectrophotometry (ND-1000; ThermoFisher Inc).

2.4. Pseudomonas relative abundance: qPCR

The sizes of the total bacterial and *Pseudomonas* communities were assessed by qPCR. Assays were conducted on a Rotor-Gene™ 6000 detection system (Qiagen). PCR chemistry, thermocycling conditions, and standard curves followed methods described in Dignam et al. (2018). Linear relationships between standard concentrations and C_T values ($R^2 > 0.99$), and amplification efficiencies above 89%, were obtained for both qPCR assays. *Pseudomonas* relative abundance was calculated as the ratio between *Pseudomonas*-specific and bacteria-specific qPCR assays. Log_{10}(*Pseudomonas*:bacteria) values were utilised in subsequent statistical analyses.

2.5. Pseudomonas community sequencing

Pseudomonas-specific 16S rRNA gene fragments were amplified using a nested PCR approach. Firstly, *Pseudomonas*-specific fragments were amplified by PCR using primers F311Ps (5′ CTGGTCTGAGAGGA TGATCAGT 3′) and R1459Ps (5′ AATCACTCCGTGGTAACCGT 3′) (Milling et al., 2005). Each 25 µl reaction mixture contained 200 nM of each primer, 1 × Bioline MyTaq reaction buffer, 1 U MyHSTaqTM DNA Polymerase (BioLine Pty Ltd.), and 2 µl (10 ng) of template DNA. PCR thermocycling conditions consisted of an initial denaturation at 95°C for 3 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The presence of amplicons of the expected size (~1150 bp) was validated by agarose gel electrophoresis. PCR products were purified using PCR paramagnetic bead solution and 96 well magnetic plate, according to the manufacturer's protocol (AxyPrep™ Mag).

Pseudomonas-specific PCR products were used as template DNA for the second (nested), general bacterial amplification. Bacteria-specific amplification and subsequent sequencing were performed at the Australian Genomic Research Facility (AGRF; Adelaide, Australia) via an established 16S rRNA gene assay using primers 341F (CCTAYGGG-RBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) with overhang adapters. Barcoded PCR amplicons were pooled and sequenced on the Illumina MiSeq platform, using Illumina's Nextera XT v2 indices and paired end sequencing chemistry.

High quality sequences were obtained from the raw dataset using mothur (v.1.36.1), based on the analysis pipeline outlined by Kozich et al. (2013). In summary: (i) contigs were assembled from forward and reverse reads; (ii) sequences that were not of the expected size (466 bp) or with ambiguous bases were removed; (iii) sequences were de-noised by pre-clustering those within 2 nucleotides of each other; and (iv) chimeras were removed using the UCHIME algorithm within mothur. Operational taxonomic units (OTUs) were clustered at 97% similarity using QIIME v.1.8.0 (Caporaso et al., 2010).

The dataset was reduced to the OTUs present in at least 4 of the 16 soils. These OTUs were taxonomically classified to genus level using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007); 96.3% were identified as *Pseudomonas*.

2.6. Soil disease suppression

The disease suppressive capacity of soils was assessed by measuring incidence and progression of *Rhizoctonia solani* AG2-1 induced damping-off of kale (*Brassica oleracea* cv. Caledonian), as described in Dignam et al. (2015). Briefly, kale seeds were sown into 7 × 7 × 8 cm pots (with sealed bases) containing 190 g of field-moist soil. Seedlings were thinned to 10 per-pot five days after sowing and the soil inoculated with *R. solani* AG2-1 isolate Rs37 seven days after sowing. In opposite corners of each pot, two 6 mm mycelial plugs of a seven-day-old 1/5 PDA *R. solani* culture were placed approximately 1 cm under the soil surface. Sterile 1/5 PDA plugs were added to the uninoculated, control treatment. Experiments were conducted in a growth room with 16 h light at 22 °C and 8 h dark at 15 °C. The pots were adjusted to 70% of the soil's maximum water holding capacity (MWHC) by watering-to-weight every other day. Randomised block experimental designs were applied to the bioassays, with three experimental replicates (inoculated and control) per field plot, and therefore, 12 experimental replicates per field treatment.

Individual plants were scored for the presence of disease expression (wirestem lesions) at two day intervals over 20 days. Disease incidence (DI) was calculated as the proportion of diseased plants per pot at the end of the assay. As a measure of disease progression, the area under the disease progress curve (AUDPC) was calculated, for each pot, from the percentage of plants with damping-off disease (two-day intervals) over the duration of the assay. Following disease assessment, fungal pathogens were isolated from the lesions of diseased plants and identified as described by Dignam et al. (2015).

2.7. Statistical analysis

2.7.1. Effect of mowing regime on soil chemistry, Pseudomonas communities and damping-off disease

Analyses of variance (ANOVA; Genstat 17) were used to test for significant effects of field treatment on soil properties, *Pseudomonas* community size and α-diversity measures, and disease progress data (AUDPC). Tukey's method of pairwise comparisons was used to test for significant differences between treatment means. Similarly, logistic regression analysis was used to test for effects of field treatment on disease incidence data (DI).

Similarity in *Pseudomonas* community composition among plant residue management treatments was calculated from the DNA sequencing OTU data using the Bray-Curtis method, following square root-transformation to down-weight the contributions of highly dominant OTUs. Permutation-based multivariate analysis of variance (PERMANOVA; 999 permutations; Anderson, 2001) was performed on the resultant dissimilarity matrix to partition the extent of variation in community composition attributable to plant residue management. Microbial species diversity (Shannon's), evenness (Pielou's) and richness (Margalef's index) were derived from *Pseudomonas* community composition data.

All multivariate analyses were performed in PERMANOVA/PRIMER7 using methods previously described (PRIMER-E Ltd., UK; Anderson et al., 2008; Clarke and Gorley, 2015)

2.7.2. Relationships between chemical, microbiological, and damping-off disease parameters

BIOENV analysis (biota and/or environmental matching; Clarke and Ainsworth, 1993) was used to find the highest rank correlation (Spearman's rho; ρ) between the community assemblage data and associated soil chemical and disease (DI and AUDPC) variables (Euclidian distance matrix). Variables were normalised to obtain 0-centred means and homogeneous variances prior to multivariate analysis. The rank correlation (ρ) indicates the amount of variation in the assemblage data that can be explained by the BIOENV-selected abiotic variables (optimised for three variables). BIOENV-derived P-values are generated from non-parametric Mantel-type testing (999 permutations; BIO-ENV; PRIMER7). Selected relationships between community assemblage data and individual soil properties were further tested using non-parametric correlation with permutation-based generation of a null-distribution to enable probability-based testing (RELATE test; PRIMER; Clarke, 1993).

Logistic and linear regression analyses were performed to determine significant relationships between soil properties and damping-off disease incidence (DI) and progression (AUDPC), respectively. Both biotic (*Pseudomonas* community abundance, diversity, evenness and richness) and abiotic (soil chemistry) variables were included in these analyses.

For all statistical analyses, draftsman's plots were generated to check for skewed soil variables prior to analysis. Potential influences of field or bioassay block structures were accounted for in the analyses; these were not significant. Differences between treatments and

relationships between variables were considered significant when $P \leq 0.05$ and marginally significant when $P \leq 0.1$.

2.7.3. DNA sequence-based and phylogenetic analyses of Pseudomonas OTUs

SIMPER analysis (similarity percentages routine; PRIMER7) was used to establish the contributions of individual OTUs to differences in *Pseudomonas* community composition between high and low disease soils. Phylogenetic analysis focused on the discriminating OTUs that were present in all 16 soils and collectively contributed ~30% (individually > 1%) to the dissimilarity between the soils.

A reference phylogenetic tree of the *Pseudomonas* genus was generated based on phylogenetic analysis of the 16S rRNA gene sequences (1347 bp) of 101 *Pseudomonas* type strains. These reference sequences were selected to provide coverage of the major groups and subgroups defined by Gomila et al. (2015). Sequences were retrieved from the Ribosomal Database Project (RDP; Wang et al., 2007) and aligned using CLUSTAL W software (Larkin et al., 2007). A maximum-likelihood tree was inferred from evolutionary distances calculated using the General Time Reversible model (Tavaré, 1986). Bootstrap analysis was based on 1000 replicates. Phylogenetic analyses were performed in the SeaView4 package. The tree was rooted using *Cellvibrio japonicus* as the out-group (Gomila et al., 2015). Without altering the base alignment of the long length reference sequences, *Pseudomonas*-specific sequences of interest (466 bp; Illumina sequencing data) were placed into the reference tree using PAGAN (Löytynoja et al., 2012).

It is important to note that the 16S rRNA gene was used to gain an understanding of the phylogenetic distribution of environmental sequences of interest. In depth phylogenetic analysis of representative isolates would require multilocus sequence analysis of multiple concatenated genes.

2.8. Accession numbers

All sequence data are available from the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra), accession number PRJNA502481.

3. Results

3.1. Effect of field treatment on the chemical composition of soil

There was a significant effect of plant residue management on 15 of the 22 soil properties measured (Table 1). Properties that differed between field treatments were predominantly related to: soil organic matter (SOM) quantity (organic matter, total carbon, total nitrogen); SOM quality (C:P and carbon recalcitrance (DOC aromatic content)); and indicators of microbial biomass and activity (AMN and AN). There was no significant variation in pH between treatments.

Measures of SOM quantity were consistently and significantly lower in the non-mown (NM) plots than in plots that were infrequently mown and the mown biomass left on the plot (IL). The effect of plant residue management on SOM quality, however, was dependent on the measure of SOM quality. The C:P ratio was significantly higher in plots in which mown biomass had been removed (IR) than in NM plots, while DOC aromatic content was significantly higher under the NM treatment than in any of the other treatments. AMN and AN were both significantly higher in plots where mown biomass was left on the plot (FL and IL) than in the NM plots.

3.2. Effect of grassland management on the microbiological composition of soil

Plant residue management significantly affected *Pseudomonas* community composition (PERMANOVA main effects test; Table 2). The influence of the field treatments was strong, accounting for nearly half the total variation in community composition (√CV; Table 2). Differences among treatments are represented in the MDS ordination (Fig. 1). *Pseudomonas* community composition in NM plots was significantly different to those from all other mowing regimes (average similarity < 55%). Similarly, IR plots were significantly different to those from all other mowing regimes (average similarity < 61%). In contrast, communities under plots in which mown biomass had been left on (FL and IL) were similar to one another, irrespective of mowing frequency (average similarity = 67.6%).

ANOVA analysis tested for an influence of plant residue management on *Pseudomonas* species diversity indices and relative abundance (Fig. 2). Microbial species diversity (Shannon's), evenness (Pielou's), and richness (Margalef) were derived from *Pseudomonas* community composition data. Plant residue management had a significant effect on *Pseudomonas* species diversity (Shannon's index; $P < 0.001$) and richness (Margalefs index; $P < 0.001$), which were significantly lower in NM plots than all other plots, and greatest in FL plots. There was reasonable evidence to suggest an effect of plant residue management on the size of the *Pseudomonas* community (relative abundance as measured by qPCR; $P = 0.087$). There was, however, no significant effect of plant residue management on species evenness.

3.3. Effect of plant residue management on soil disease suppression

There were significant differences in disease incidence (DI) and progression (AUDPC) between soils collected from under different plant residue management (Fig. 3). Both DI and AUDPC were significantly higher in the non-mown plots (NM) than in those that had been mown frequently and the mown biomass left (FL). Disease incidence in IL soil did not differ significantly from any of the other field treatments (Fig. 3a), while, similarly to FL soil, disease progression (AUDPC) was significantly lower in IL soil than NM soil (Fig. 3b).

Background soil disease pressure was present in all treatments; damping-off disease symptoms (wirestem lesions) developed on seedlings grown in uninoculated soils collected from under each of the mowing regimes (Fig. 3). Background disease pressure did not differ significantly with plant residue management. Compared to the uninoculated soil, disease incidence and progression were significantly higher in inoculated soil for each of the field treatments. Causative agents were isolated from the lesions of both control and inoculated plants, and characterised by microscopy and sequencing of the ITS1-5.8S-ITS2 region (Dignam et al., 2015). DNA analysis of the fungi isolated from the lesions of uninoculated plants identified common soil-borne fungal species including *Trichoderma harzianum*, *Fusarium oxysporum*, and *Boeremia exigua*, in addition to *Rhizoctonia solani*. In contrast, fungi isolated from the lesions of inoculated plants were identical in nucleotide sequence to introduced *R. solani* AG2-1.

3.4. Relationships between soil chemistry, Pseudomonas communities, and damping-off disease

3.4.1. Soil chemical properties linked to differences in Pseudomonas community structure and size

DOC aromatic content, by itself, explained 80% of the variation in *Pseudomonas* community structure between field plots (RELATE; $P = 0.01$). Incorporation of additional soil properties (calcium and sodium) into the BIOENV model only explained a further 4% of the variation. Furthermore, negative correlations with DOC AC accounted for 51% and 42% of the variation in *Pseudomonas* species diversity and richness between field plots, respectively (linear regression; $P < 0.007$). However, the relationships between DOC AC and *Pseudomonas* abundance and evenness were not significant.

3.4.2. Soil chemical and microbial properties linked to differences in disease incidence and progression

Soil and microbial properties that correlated significantly, or

Table 1
The effect of plant residue management on soil chemical parameters. Field treatments consisted of four mowing regimes: mown frequently and biomass left (FL); mown infrequently and biomass left (IL); mown infrequently and biomass removed (IR); never mown (NM). Analysis of soil chemistry was conducted on one composite sample per plot and data presented are means of four replicate plots per treatment. In each column, letters in superscript indicate significant differences in individual soil variables between treatments ($P \leq 0.05$, Tukey).

	Organic matter (%)	Total C (%)	Total N (%)	Total P (mg kg^{-1})	C:N	C:P	HWEC[a] (ppm)	DOC[b] aromatic content (Abs254)	AMN[c] (μg g^{-1})	AMN:N	AN[d] (kg ha^{-1})
FL	6.13^{ab}	3.53^{ab}	0.33^{ab}	564.8^{a}	11.02	0.0063^{ab}	195.3^{ab}	0.84^{a}	64.75^{a}	2	93.50^{a}
IL	6.48^{b}	3.75^{b}	0.35^{b}	629.2^{a}	10.75	0.0060^{ab}	210.7^{b}	0.83^{a}	68.50^{a}	1.93	95.75^{a}
IR	5.45^{a}	3.15^{a}	0.30^{ab}	465.8^{b}	10.5	0.0068^{b}	150.2^{a}	0.77^{a}	56.00^{ab}	1.88	82.50^{ab}
NM	5.23^{a}	3.03^{a}	0.27^{a}	590.5^{a}	11.25	0.0051^{a}	203.6^{b}	1.10^{b}	42.50^{b}	1.58	64.75^{b}

	pH	Olsen P (mg L^{-1})	TBS (%)	CEC[e] (me $100g^{-1}$)	Volume Weight (g ml^{-1})	Al (mg kg^{-1})	K (me $100g^{-1}$)	Mg (me $100g^{-1}$)	Ca (me $100g^{-1}$)	Na (me $100g^{-1}$)	Soil Moisture (%)
FL	5.58	38.75^{a}	56	17.5	0.97^{ab}	1.10^{a}	1.10^{a}	1.96^{a}	6.58	0.178^{ab}	11.42
IL	5.6	41.25^{a}	53.75	18.25	0.94^{a}	1.20^{ab}	1.07^{a}	1.99^{a}	6.65	0.188^{b}	11.56
IR	5.63	15.25^{b}	51.25	16.5	0.98^{bc}	1.98^{ab}	0.25^{b}	1.57^{b}	6.43	0.255^{c}	11.88
NM	5.68	38.50^{a}	51.25	16.5	1.01^{c}	2.20^{b}	0.95^{a}	1.79^{ab}	5.6	0.138^{a}	11.91

[a] Hot water extractable carbon (HWEC).
[b] Dissolved organic carbon (DOC) aromatic content; higher DOC AC indicates greater carbon recalcitrance.
[c] Anaerobically mineralisable nitrogen (AMN).
[d] Available nitrogen (AN).
[e] Cation exchange capacity (CEC).

Table 2
Influence of mowing regime on *Pseudomonas* community structure. From a similarity matrix (Bray Curtis) of *Pseudomonas* community assemblage, derived from Illumina sequencing OTU data, permutation-based multivariate ANOVA was used to test for treatment structure. Plant residue management was the fixed factor in the PERMANOVA design and pairwise comparisons detected differences between individual treatments. √CV is the square-root of the component of variation (Anderson et al., 2008), which provides a measure of the size of effect for each component in the analysis. P-values were derived from permutation testing (999 times; PERMANOVA).

PERMANOVA	Factor	Pairwise comparison	√CV	P-value
Main Test	Plant residue management		23.46	**0.0001**
	Residual		25.62	
Pairwise Test	Plant residue management	FL vs IL		0.31
		FL vs IR		**0.029**
		FL vs NM		**0.029**
		IR vs IL		**0.026**
		IR vs NM		**0.029**
		IL vs NM		**0.03**

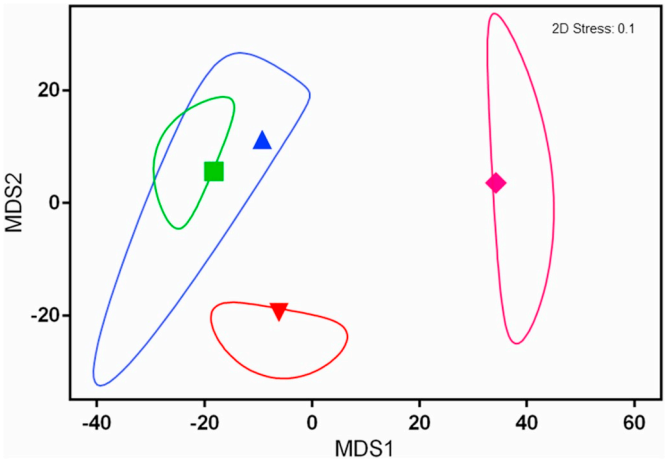


Fig. 1. Influence of plant residue management on *Pseudomonas* community structure: non-metric MDS ordination plots. Mean communities (individual points) for each field treatment were derived from 150 bootstrap averages; frequently mown/biomass left (FL; ▲), infrequently mown/biomass left (IL; ■), infrequently mown/biomass removed (IR; ▼), and never mown (NM; ◆). Clouds surrounding individual points are 95% region estimates for the mean communities, and represent the spread of the bootstrap averages. Field treatments for which *Pseudomonas* community composition is similar, individual points and/or 95% region estimates are in closer proximity. Observations are statistically supported by PERMANOVA testing of Bray-Curtis dissimilarity data (Table 2).

marginally significantly, with disease incidence or progression are given in Table 3. Notably, as *Pseudomonas* species diversity or richness increased, disease incidence (DI) and progression (AUDPC) decreased (Table 3).

Pseudomonas species diversity and richness individually explained approximately 30% of the variation in disease progression across field treatments, and were mutually exclusively included as the first parameter in stepwise regression models for DI and AUDPC (data not shown). AUDPC was also positively correlated with DOC AC and negatively correlated with total N, which accounted for 28.6% and 28.7% of the variation in disease progression, respectively. The relationships between organic matter, total C, volume weight, and AUDPC were only marginally significant and individually accounted for less than 25% of the variation in disease progression. Correlations with DI were not significant for any other edaphic or microbiological properties.

3.4.3. High vs low disease soils: soil chemistry and microbiology

Measures of both damping-off disease incidence (DI) and progression (AUDPC) on kale were significantly lower in FL soils than in NM soils. FL and NM soils were subsequently termed 'low' and 'high' disease potential soils, respectively, and significant differences in disease suppression, *Pseudomonas* communities, and soil chemistry between these soils are summarised in Table 4. Of these, *Pseudomonas* species richness and DOC AC were also positively correlated with disease measures across all field treatments (Table 3). Furthermore, indicators of microbial biomass and activity (AMN and AN) differed between these soils.

Pseudomonas community structure was also shown to differ significantly between low (FL) and high (NM) disease soils (Fig. 1); the average dissimilarity was 42.8% (PERMANOVA). SIMPER analysis was conducted to determine which OTUs were contributing to the dissimilarity in *Pseudomonas* community between low and high disease soils. Fourteen *Pseudomonas* OTUs were identified that collectively accounted

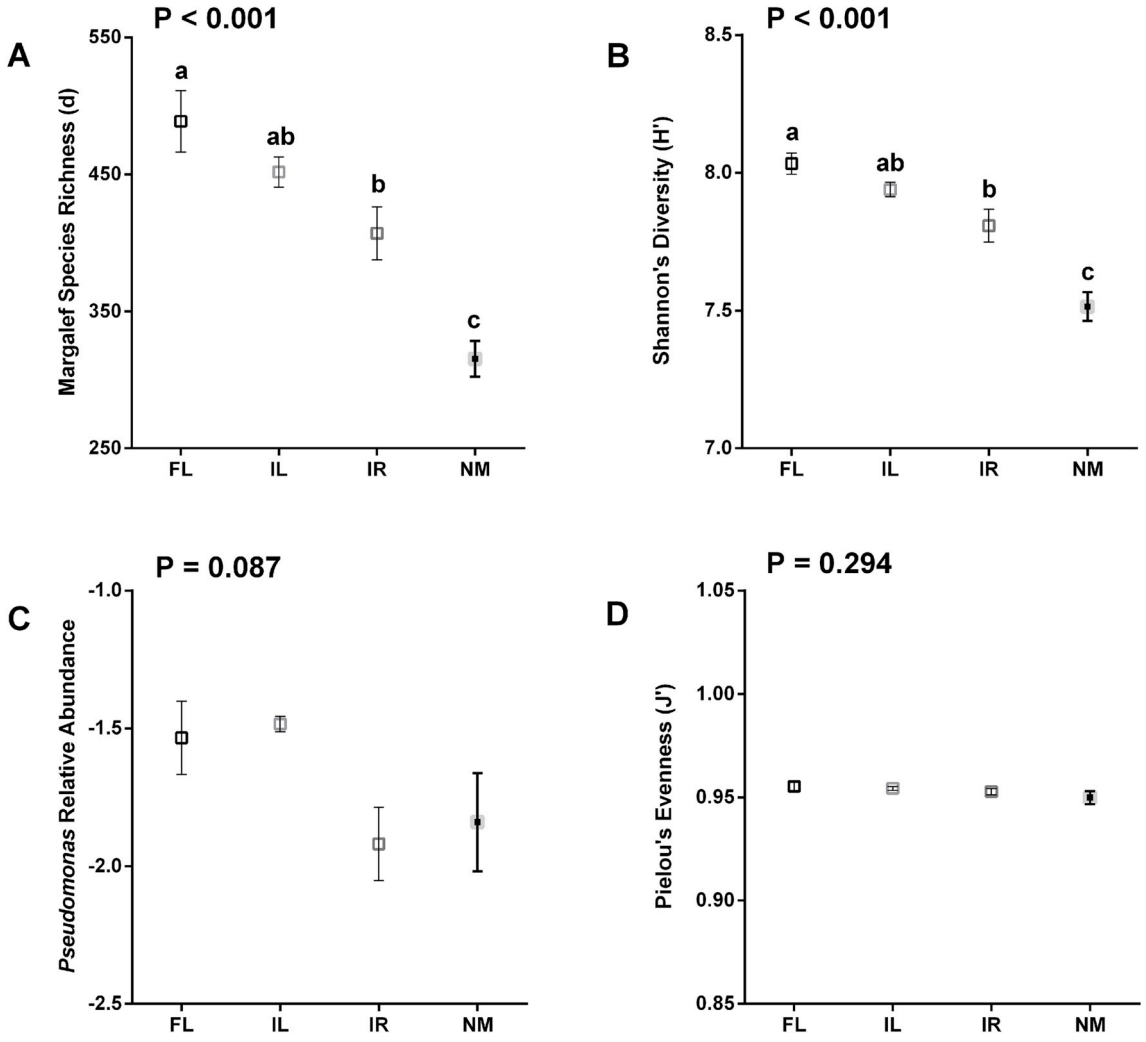


Fig. 2. The effect of plant residue management on *Pseudomonas* species richness (A), species diversity (B), relative abundance (C), and species evenness (D). Data presented are means of 4 plots per treatment ± SEM. P values were derived from ANOVA (Genstat). Letters above data points indicate significant differences between treatments ($P \leq 0.05$; Tukey).

for ~30% of the difference between these two soils (see Supplementary data Appendix A, Table S1). Of these, 11 were higher in abundance in low disease soils and three were higher in abundance in high disease soils.

Representative 466 bp 16S rRNA gene sequence fragments for each of the 14 OTUs were included in taxonomic analysis, along with sequences of 101 selected species (type strains) representative of the major groups and subgroups within the genus (Fig. 4). All 14 OTUs belonged to the *Pseudomonas* genus but were phylogenetically dispersed among sub-groups, i.e. they did not form a single clade. Furthermore, OTUs higher in abundance in one or other soil did not cluster together.

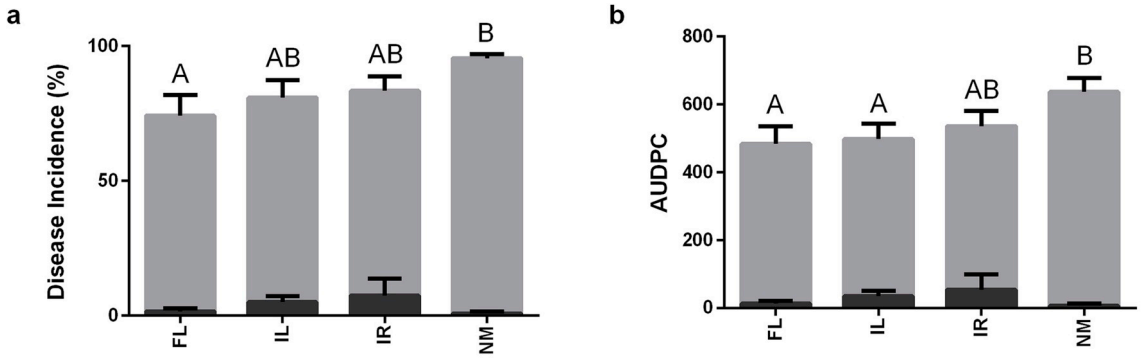


Fig. 3. *Rhizoctonia solani* Rs37 damping-off disease incidence (a) and progression (b) on kale seedlings (mean ± SEM, N = 12). Means are compared between four plant residue management field treatments: frequently mown/biomass left (FL), infrequently mown/biomass left (IL), infrequently mown/biomass removed (IR), and never mown (NM). Damping-off disease progress in uninoculated pots (dark grey) is represented as a proportion of disease progress in the inoculated treatments (light grey). Letters above the bars indicate significantly different inoculated treatments ($P \leq 0.05$; Tukey). Disease incidence and progression did not differ significantly between uninoculated treatments.

Table 3
Relationships between *R. solani*-induced damping-off disease incidence (DI) and progression (AUDPC) on kale and soil chemical and microbiological properties. Soil properties for which either logistic regressions with DI, or linear regressions with AUDPC, were significant ($P \leq 0.05$) or marginally significant ($P \leq 0.1$) are presented. R^2 values are given for significant or marginally significant correlations and indicate the percentage of variation accounted for by the regression. Slopes of individual regressions describe the magnitude and direction between a particular soil variable and AUDPC.

Regression Analysis	DI		AUDPC		
	P-Value	R^2	P-Value	R^2	Slope
Soil chemical and physical properties					
Organic Matter	0.268		*0.079*	*20*	*−89.2*
Total Carbon	0.281		*0.081*	*20*	*−151.6*
Total Nitrogen	0.174		**0.032**	**28.7**	**−176.1**
Volume Weight	0.382		*0.057*	*24*	*1698*
DOC AC[a]	0.167		**0.033**	**28.6**	**485**
Soil microbiological properties					
Pseudomonas species diversity	*0.071*	*15.8*	**0.026**	30.6	−325
Pseudomonas species richness	**0.035**	**22.8**	**0.023**	**31.8**	**−0.932**
Pseudomonas relative abundance	0.115		*0.09*	*19*	*−185*

[a] Dissolved organic carbon aromatic content (DOC AC); higher DOC AC indicates greater carbon recalcitrance.

Table 4
Low vs high disease soils: differences in soil chemistry and microbiology. Soil properties shown to differ significantly between FL (low disease) and NM (high disease) soils are presented (ANOVA; Tukey's; $P \leq 0.05$). Data are mean values ± standard error derived from 4 replicate field plots (*Pseudomonas* and soil chemistry data) or 12 bioassay replicates (DI and AUDPC).

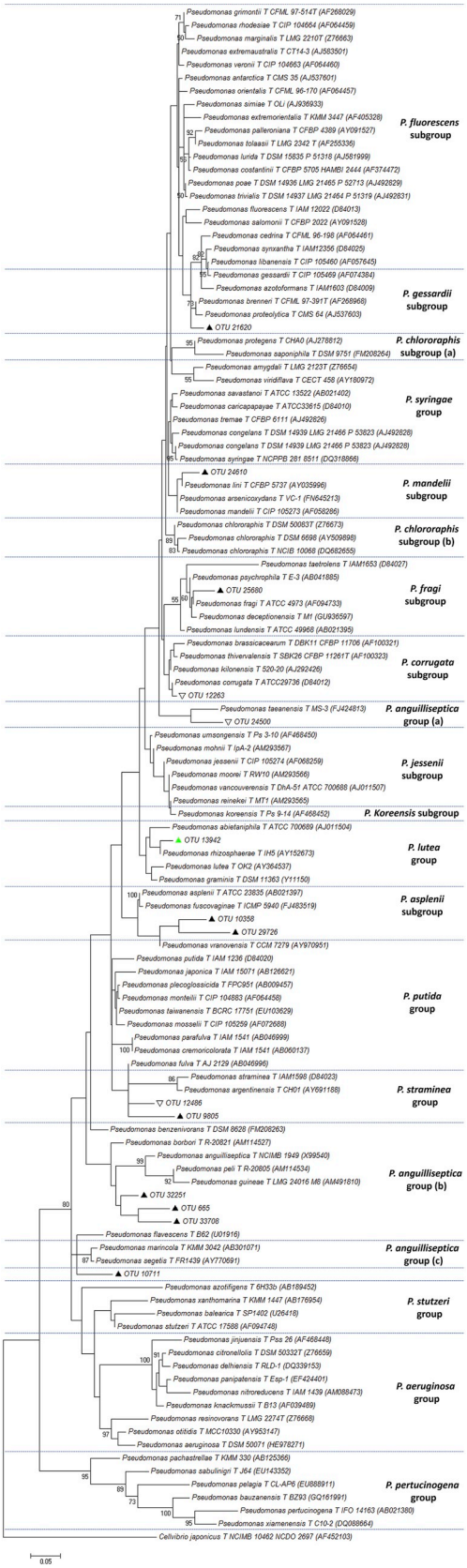
	Low Disease (FL)	High Disease (NM)
DI	74.17 ± 26.29	95.45 ± 4.98
AUDPC	483.75 ± 183.35	637.08 ± 134.14
Pseudomonas community composition	Average similarity < 43%	
Pseudomonas species diversity	8.03 ± 0.08	7.51 ± 0.10
Pseudomonas species richness	488.75 ± 44.76	315.38 ± 25.99
DOC AC[a]	0.84 ± 0.04	1.10 ± 0.10
AMN[b]	64.75 ± 9.91	42.5 ± 9.26
AN[c]	93.5 ± 11.39	64.75 ± 13.82
Extractable aluminium	1.1 ± 0.14	2.2 ± 0.98
Volume Weight	0.97 ± 0.04	1.01 ± 0.03

[a] Dissolved organic carbon aromatic content (DOC AC); higher DOC AC indicates greater carbon recalcitrance.
[b] Anaerobically mineralisable nitrogen (AMN).
[c] Available nitrogen (AN).

OTU 13942 is of particular interest, alone contributing 7.4% to the total dissimilarity in *Pseudomonas* community structure between low and high disease soils, and higher in abundance in low compared to high disease soils. This OTU clustered close to *Pseudomonas rhizosphaerae*, within the *Pseudomonas lutea* group (Fig. 4).

4. Discussion

This study aimed to test the impact of plant residue management on soil chemical and biological properties, in particular disease suppression. In comparison to non-mown plots, long-term, frequent amendment with plant litter (mown biomass) altered the chemical, physical, and microbiological parameters of grassland soils. These changes were associated with enhanced soil disease suppression. *R. solani*-induced damping-off disease incidence and severity on kale plants was significantly lower in frequently mown plots in which plant litter was retained (FL) in comparison to those plots that were not mown (NM) for the 20-year duration of the field trial. Notably, *Pseudomonas* bacterial community diversity and richness, soil organic matter content and carbon recalcitrance correlated with disease progression.



(caption on next page)

Fig. 4. Maximum-likelihood tree inferred from the 16S rRNA gene sequences of 101 *Pseudomonas* type strains (1347bp) and 14 *Pseudomonas* OTUs (466bp; Ilumina sequencing data). Type strain accession numbers are given in parenthesis of individual nodes. Percentage bootstrap values (numbers at nodes) are based on 1000 replicates. The scale bar indicates a phylogenetic distance of 0.05 nt substitutions per site. Trees were rooted using *Cellvibrio japonicas* as the out-group (Gomila et al., 2015). The 14 *Pseudomonas* OTUs included in the analysis are those that collectively contributed ~30% to the dissimilarity between low (FL) and high (NM) disease soils. Of these OTUs, 11 were higher in abundance in low disease soils (filled triangle) and 3 were higher in abundance in high disease soils (empty triangle). *Pseudomonas* OTU 13942 (filled green triangle) contributed 7.4% to the total dissimilarity in *Pseudomonas* community structure between low and high disease soils, and was higher in abundance in low compared to high disease soils. Groups and subgroups are defined based on those derived from multilocus sequence analysis (Gomila et al., 2015). For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.

4.1. Grassland management affects soil chemistry

Grassland management of plant litter and senesced vegetation, through implementation of varied mowing regimes, has led to alterations in soil chemical and physical parameters at this long-term field trial site. Of these parameters, SOM quantity (organic matter, C and N), carbon recalcitrance (DOC aromatic content), and volume weight (bulk density) were correlated with damping-off disease progression (AUDPC). Measures of SOM quantity were consistently higher in plots with mown biomass retained than plots that were not mown or where biomass was removed (naturally, statistical support of treatment effects varied among soil properties), while carbon recalcitrance was greater in non-mown plots than in any other treatment.

Changes in soil chemical characteristics are likely attributed to the differences in the retention and subsequent microbial decomposition of organic matter, and the recycling of nutrients associated with retention of plant litter (Simpson et al., 2012; Adair et al., 2013). While similarly to our study, Simpson et al. (2012), sampling at this site in Autumn 2009, found effects of mowing regime on total C and total N but not C:N, while Adair et al. (2013), sampling in Spring 2011, found significant differences in C:N between mowing regimes but not in total C or total N. Such temporal variation is potentially attributable to differences in the stage or rate of decomposition, as affected by seasonal fluctuations in environmental moisture and temperature. It is also likely that changes in abiotic parameters, in particular carbon availability, are driven by litter quality and below-ground changes in root exudation. In addition to regular inputs of plant litter, mowing has been shown to increase rhizodeposition of labile C (Hamilton and Frank, 2001). Furthermore, litter quality (Bonanomi et al., 2013) and root exudate patterns (Berg et al., 2009) are known to be plant species specific, and over the 20 year duration of the field trial, botanical composition has changed considerably and now varies between field treatments (Materials and Methods; Adair et al., 2013). Following 17 years at this trial, cocksfoot (a perennial grass) was dominant and clover absent in non-mown (NM) plots, while white clover was a dominant plant species in all mown plots (IL, IR and FL). Although plant species composition was not recorded in the year of sampling for this study, anecdotal evidence suggests that clover cover was similar to levels measured at 17 yrs. In comparison to grasses, clovers produce high quality (low C/N ratio) above- and belowground biomass (Dijkstra et al., 2006; van Eekeren et al., 2009). Therefore, it is likely that the shift in above-ground composition has resulted in frequent additions of plant litter rich in labile carbon to FL plots, and organic material more resistant to decomposition in NM plots, contributing to the below-ground changes in soil chemistry.

4.2. Carbon availability is the primary driver of management-induced shifts in Pseudomonas community structure

Management-dependent shifts in *Pseudomonas* community structure were observed (Fig. 1), and corresponding shifts in *Pseudomonas* species diversity and richness were correlated with damping-off disease incidence and severity (Table 3). *Pseudomonas* species diversity and richness were highest in frequently mown plots with biomass retained and lowest in non-mown plots. In contrast, Adair et al. (2013) found no effect of field treatment on the richness or diversity of the total bacterial community at this site. Similarly to bacterial communities, *Pseudomonas* communities have been shown to be responsive to management practices in both agricultural and grassland soils (Garbeva et al., 2004; van Overbeek et al., 2012; Wakelin et al., 2012). However, in a previous assessment of New Zealand agricultural grassland soils, *Pseudomonas* communities were found to be driven by edaphic properties distinct from those driving changes in the total bacterial community (Dignam et al., 2018). Furthermore, Goldfarb et al. (2011) observed differential responses of individual taxa within the soil bacterial community to organic amendments of varying carbon recalcitrance. Therefore, it is not surprising that the drivers of diversity for a single genus, *Pseudomonas* in this case, differ from those affecting the wider bacterial community.

Pseudomonas community composition differed between all plant residue management regimes, with the exception of plots in which mown biomass was retained (FL and IL); these plots supported similar communities. Adair et al. (2013) observed the same pattern in separation of total bacterial communities by field treatment at this site, with shifts in plant community composition and Olsen P explaining the greatest proportion of the variation in bacterial communities. Carbon recalcitrance (DOC aromatic content) was responsible for 80% of the variation in *Pseudomonas* community composition between treatments. The influence of carbon recalcitrance was closely linked with changes in *Pseudomonas* species diversity and richness, but not evenness or abundance. Inverse correlations with DOC aromatic content explained up to 51% of the variation in *Pseudomonas* species diversity and richness. In a previous study of agricultural grasslands, we also found the size and structure of *Pseudomonas* communities to be sensitive to soil organic matter quality (Dignam et al., 2018). Furthermore, *Pseudomonas* communities have been shown to be sensitive to the soil DOC fraction under grass-clover leys (van Overbeek et al., 2012), and at a broader taxonomic level, the relative abundance of *Pseudomonadales* was shown to respond to soil amendment with sources of labile C (Goldfarb et al., 2011). Many *Pseudomonas* spp. are assumed to preferentially consume labile C (Smit et al., 2001) and thus, we hypothesised that *Pseudomonas* species diversity would increase with decreasing carbon recalcitrance (increasing C availability). Our findings support this hypothesis, with both Shannon's diversity and Margalef species richness increasing with carbon availability. Similarly, using DNA fingerprinting, van Overbeek et al. (2012) found that *Pseudomonas* species diversity was highest when the soil DOC fraction (carbon availability) was highest following two years of grass-clover ley.

4.3. Increased Pseudomonas species diversity and richness are associated with enhanced soil disease suppression

The incidence and severity of *R. solani* induced damping-off disease on kale seedlings was significantly lower in frequently mown/biomass retained soils than in non-mown soils. Of the soil properties assessed, *Pseudomonas* species diversity and richness were identified as the primary parameters explaining the greatest proportion of variation in the suppressive capacity of soils across mowing regimes. This supported our hypothesis that management-induced changes in *Pseudomonas* communities would be indicative of changes in soil susceptibility to a broad host range pathogen; increased species diversity and richness were associated with reduced disease levels.

Our findings are in line with previous field studies that have also related increased suppression of *R. solani*-induced disease to increased *Pseudomonas* diversity in response to contrasting management practices (Garbeva et al., 2004; van Overbeek et al., 2012). While the inherent complexity of field and environmental studies make it difficult to accurately define the mechanisms that underlie the positive relationship between *Pseudomonas* diversity and disease suppression, valuable insights have been gained from detailed studies utilising simplified model systems and constructed communities. For example, more diverse constructed *Pseudomonas* communities have been shown to exhibit the following traits: enhanced production of the antifungal secondary metabolite DAPG and suppression of soil-borne fungal pathogens (Jousset et al., 2014); enhanced resource competition in the rhizosphere, reduced pathogen density and lower plant disease incidence (Hu et al., 2016); and enhanced N mineralisation leading to the amplification of positive plant-soil feedback (Weidner et al., 2015). Furthermore, Garbeva et al. (2011) demonstrated that secondary metabolite production by *Pseudomonas fluorescens* Pf0-1 was stimulated in response to competition for nutrients with phylogenetically diverse bacteria.

The negative correlation we have observed between *Pseudomonas* species diversity/richness and carbon recalcitrance, and the significant effect of species diversity, richness, and DOC aromatic content on disease progression, suggests that higher carbon availability has selected for a more species rich and potentially more competitive and suppressive community. The observed shifts in *Pseudomonas* community structure, underpinned by chemical changes associated with plant residue decomposition and rhizodeposition, could be reflective of similar shifts in the wider microbial community to, for example, a more copiotrophic and competitive community. Fungal:bacterial ratios have previously been shown to be higher in unmanaged grasslands (Bardgett et al., 1996); similar shifts in community composition in the non-mown plots at this site may have resulted in a more oligotrophic, fungal-dominated community.

General disease suppression is attributed to the competitive activity of the total soil microbiota (Weller et al., 2002), and is the assumed mechanism underlying the suppressive capacity of organic amendments (Hiddink et al., 2005). The application of organic amendments to soil has been shown to effectively control a range of soilborne pathogens, including *Gaeumannomyces graminis* var *tritici*, *Rhizoctonia solani*, *Thielaviopsis basicola*, *Verticillium dahliae*, *Fusarium* spp., *Pythium* spp., and *Phytophthora* spp. (Hoitink and Boehm, 1999; Bonanomi et al., 2010, 2018). Furthermore, although the effects of organic amendments on disease suppression can be inconsistent, measures of microbial activity, rather than soil chemical parameters, were found to more accurately predict the disease suppressive potential of organic amendments (Bonanomi et al., 2010). Although not significantly correlated with disease levels across all mowing regimes, AMN and AN, representative of soil microbial activity, were significantly higher in low disease soils than high disease soils (Table 4). Low disease soils received more frequent additions of organic matter, and typically, soils with higher organic matter turnover have shown enhanced suppression of *Rhizoctonia*-induced diseases through increased microbial activity and altered microbial composition (Stone et al., 2004). Similarly, Bonanomi et al. (2017) demonstrated that conditioning soil with frequent additions of organic matter enhanced microbial activity and functional diversity, and increased the fungistasis response of soil to fungi from a range of ecological niches. Organic matter-mediated general suppression of soil-borne fungal pathogens is supported by multiple mechanisms. In diverse soil communities, a range of disease suppressive functions, including nutrient competition, hyperparasitism, antibiosis, and induced systemic resistance contribute to disease suppression and plant protection (Haas and Défago, 2005; Bakker et al., 2007; Raaijmakers and Mazzola, 2012). In a recent study, the application of the environmental microarray GeoChip demonstrated that functional genes with putative roles in a range of disease suppressive mechanisms were widely distributed in agricultural grasslands, and that the abundance of these genes increased with carbon availability (Dignam et al., 2018).

In comparison to DNA fingerprinting approaches, the *Pseudomonas*-specific Illumina sequencing-based approach utilised in this study allowed a more in-depth analysis of the OTUs identified as distinguishing between low and high disease soils. Phylogenetic assessment of these OTUs showed that they were dispersed among phylogenetic groups within the *Pseudomonas* genus, indicating that a diverse range of *Pseudomonas* species were contributing to the separation of communities between low and high disease soils. Within the *Pseudomonas* genus, the suppression of soil-borne diseases has typically been attributed to antagonistic fluorescent species enriched in the rhizosphere (Haas and Défago, 2005). Thus, we may expect that OTUs enhanced in low disease (suppressive) soils would cluster within such clades. However, Hiddink et al. (2005) found that the abundance of fluorescent *Pseudomonas* spp. was reduced in suppressive, organically managed soils in comparison to conventionally managed soils. Furthermore, there is growing evidence to support the role of consortia of indigenous soil microbes in disease suppression (for example, Mendes et al., 2011; Penton et al., 2014). Metabolic functions associated with suppressive activity, such as the production of antibiotic metabolites, are not unique to a narrow group of *Pseudomonas* bacteria, but are instead harboured by a diverse range of bacterial and fungal taxa (Raaijmakers and Mazzola, 2012). Even within a species, individual strains can harbour distinct and diverse sets of biocontrol traits (Loper et al., 2012). In this study, the taxonomic, and potentially functional, diversity within the *Pseudomonas* community of suppressive (low disease) soils is likely reflective of the contribution of a diverse and competitive microbiota to general, rather than specific, disease suppression.

The single OTU that contributed 7.4% to the dissimilarity in *Pseudomonas* community structure between high and low disease soils (OTU 13942; Fig. 4) was higher in abundance in low disease soils. Phylogenetic assessment placed this OTU in the vicinity of *Pseudomonas graminis* (Behrendt et al., 1999), *Pseudomonas rhizosphaerae* (Peix et al., 2003) and *Pseudomonas lutea* (Peix et al., 2004), within the *P. lutea* group defined by Gomila et al. (2015). These *Pseudomonas* species, isolated from the phyllosphere (*P. graminis*) and rhizosphere (*P. rhizosphaerae* and *P. lutea*) of grass species, form a phenotypic group within the *Pseudomonas* genus that do not produce oxidase or fluorescent pigments, and exhibit broad spectrum utilisation of carbon sources (Peix et al., 2004). Furthermore, there is evidence to suggest that isolates of *P. graminis* express antagonistic traits (Alegre et al., 2013), although the biocontrol potential of isolates of these species against soil-borne fungal pathogens has not yet been assessed. Perennial ryegrass is a predominant component of the plant community in these field plots (Adair et al., 2013). Interestingly, the diversity and composition of grassland plant species has been shown to differentially affect the abundance of antagonistic *Pseudomonas* spp. (Latz et al., 2012, 2016). Frequent mowing with retained biomass would lead to continuous addition of above-ground grass biomass with associated microbes, and also to alteration of root exudation patterns (Hamilton and Frank, 2001), to which rhizosphere bacteria, particularly Pseudomonads, have been shown to be sensitive (Drigo et al., 2009). Although functional traits can be phylogenetically dispersed, based on sequence homology, we may expect isolates representative of this OTU to harbour similar phenotypic characteristics to members of the group within which it is placed (Fig. 4). In order to gain a mechanistic understanding of how representative isolates of this OTU may contribute to the suppressive capacity of these soils, further culture-dependent analysis of *Pseudomonas* species would be required.

It is worth noting that across all treatments a negative correlation with total N individually explained 29% of the variation in disease progression; measures of soil total N and available N were highest in the most suppressive (low disease) soil. While excess soil nitrogen has been associated with the survival of fungal pathogens in soil and increased levels of *R. solani*-induced disease (Papavizas, 1970), our results suggest that direct suppression of pathogen growth through reduced soil N is

not a mechanism contributing to disease suppression in these soils. Simultaneously to the plant-pathogen bioassay, we conducted a toothpick assay (Paulitz and Schroeder, 2005), which confirmed the spread of the pathogen through soil in the absence of a plant host (data not shown). This further confirmed that soil suppressiveness across plant residue treatments was attributed to management-induced shifts in microbial communities, driven through changes in soil chemistry, rather than pathogen suppression by soil chemical or physical parameters.

5. Conclusions

Collectively, our results suggest that management-induced shifts in *Pseudomonas* community composition, notably species diversity and richness, provide a better indicator of soil conduciveness to disease caused by the broad host range pathogen (*R. solani*) than soil chemical parameters. Increased soil organic matter content, carbon availability, *Pseudomonas* species diversity and microbial activity distinguished low disease soils from high disease soils. Such changes are indicative of a general mechanism of disease suppression attributed to the competitive activity of the total microbiota. In response to management practices that alter soil organic matter quality, shifts in *Pseudomonas* community structure served as a taxonomic indicator of soil suppressive potential. Whether *Pseudomonas* bacteria provide a 'universal' indicator of suppressive change across management practices and pathosystems remains to be tested.

Importantly, we demonstrate that management practices that result in the frequent addition of organic residues to grassland soils enhance the diversity and activity of plant-beneficial bacterial taxa. The application of such practices in pastoral agriculture may provide a sustainable approach to limiting the impact of soil-borne diseases and enhancing plant productivity.

Declaration of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.05.020.

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