Metaproteomics is an emerging technique for directly assessing cellular function and interactions within an environment. In complex environments such as soil, there is a vast dynamic range of microbial species abundance and protein expression levels. Data acquisition is biased towards high-abundance proteins, for example, chaperonins, ribosomal proteins, elongation factors and ATP synthases (Benndorf et al., 2007; Dill et al., 2010). Removal of these intracellular proteins could allow access to functionally important low-abundance proteins in the soil enzyme pool and at the interface of cell and soil, the soil metaexoproteome.

Chitin provides one of the dominant sources of organic nitrogen in soil (Gooday, 1990) and chitinases are implicated in its mineralization in a wide range of contexts (Rhazi et al., 2000; Muzzarelli, 2011), especially in nitrogen-poor soils (Olander and Vitousek, 2000). The molecular diversity of chitinases in soil microbial communities has been studied (Williamson et al., 2000; Metcalfe et al., 2002; Hjort et al., 2010) but very few have focused on the functional contributions of members of the chitinolytic bacterial community. We report here the first attempt to recover and analyse extracellular proteins in soil adopting a novel approach to extract the metaexoproteome. Our data indicate that one actinobacterial group was disproportionately responsible for chitin breakdown. Soil was sampled from an island off the north coast of Cuba known for its high biodiversity and wide range of chitinolytic bacteria (Williamson et al., 2000; Williamson, 2001). Microcosms were constructed and amended with 1% crude crab shell (α-chitin) or squid pen (β-chitin) to enrich the microbial community, an unamended control was included for the 16S rRNA gene metataxonomic analysis (Supplementary Method S1). Community DNA was extracted and sequenced on a 454 GS FLX instrument with titanium reagents (Roche, Basel, Switzerland) using eubacterial primers Gray28F and Gray519R (Dowd et al., 2008) and GH18 Group A chi primers GASQF and GASQR (Williamson et al., 2000); the data were analysed with the bioinformatics package QIIME (Caporaso et al., 2010) (Supplementary Method S2). The metaexoproteome extraction is a modification of Masciandaro et al. (2008). In brief, 100 g soil was gently agitated with a K2SO4-based extraction solution (1:3 w/v) then the solid fraction and cells removed by centrifugation and filter sterilization before dilution (3:1 v/v) with 18.2 MΩ cm water and dialysis overnight. The retentate was concentrated to a final volume of ~1 ml by ultrafiltration and using a centrifugal concentrator for direct loading onto a one-dimensional SDS–polyacrylamide gel electrophoresis gel. Gel-dependent nanoflow liquid chromatography-tandem MS (nanoLC-MS/MS) analysis was
performed and the resultant Micromass peak list files interrogated with the NCBI mr database using the MASCOT search engine (Matrix Science, London, UK). The full list of proteins was filtered to remove the few eukaryotic proteins and hits with <2 significant unique peptides (Supplementary Methods S3 and S4).

To successfully target the metaexoproteome, cell integrity must be maintained. Minimal cell lysis during the extraction was demonstrated experimentally by spiking soil with *Escherichia coli* over-expressing His-tagged phosphoribosyl isomerase A in the cytoplasm and attempting to detect the His-tag in the extract by western blot (Supplementary Method S5), as no protein was detected we believe the method did not lyse cells. The majority of 52 recovered proteins were Gram-negative in origin and attributed to the extracellular fraction or outer membrane (Supplementary Tables S1 and S2). Across both amendments, 73% of proteins were predicted to have a signal peptide (Nielsen et al., 1997), 13% to have transmembrane helices (Sonnhammer et al., 1998; Krogh et al., 2001), 17% to be TRAP transporters and 52% to be ABC transporters. These features are suggestive of export or being membrane bound and indicate that the metaexoproteome is representing the functional interface between cell and environment.

In vitro secretomes commonly feature a similar range of TRAP and ABC transporters in addition to selected extracellular enzymes depending on the enrichment (Adav et al., 2010; Christie-Oleza and Armengaud, 2010; Christie-Oleza et al., 2012). The only extracellular enzymes identified were chitinases.

Recovered proteins were affiliated with three phyla, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*, this correlated well with the 16S rRNA gene data set (Figure 1). Only two genera dominated the metaexoproteome, both in terms of number of proteins recovered and protein abundance measured by emPAI (Ishihama et al., 2005), the actinomycete *Nocardiopsis* and the rhizobiale *Nitratireductor*. Approximately 17% of the identified proteins were matched to *Nocardiopsis*. The family *Nocardiopsaceae* was undetected in the unamended 16S rRNA gene data set but was one of the few actinobacterial groups to increase in abundance with α-chitin amendment, accounting for 3.7% of the community.

The majority of proteins were related to the transport and metabolism of amino acids, carbohydrates and inorganic ions, namely phosphate and phosphonate. Two GH18 chitinases were identified by peptides from within their catalytic domains, ChiA from *Nocardiopsis lucentensis* and *N. dassonvillei* (Supplementary Table S1). Corresponding *Nocardiopsis chiA*-like sequences were identified in the GH18 chi gene pyrosequencing data set (Figure 1). *Nocardiopsis* chitinases have been shown to have chitinolytic activity against α- and β-chitin (Tsujibo et al., 2003) and to be capable of fast and complete degradation of crystalline chitin in liquid media (Sorokin et al., 2012).

A fluorogenic chitinase assay (Sigma-Aldrich, St Louis, MO, USA) was performed on the extracts from α-chitin-amended microcosm soil and metaexoproteome (Supplementary Method S6). Both extracts showed activity against the monomeric substrate but the metaexoproteome extract had proportionally higher activity against the more representative di-NAG and tri-NAG substrates. It is probable that the chitinase activity detected in the metaexoproteome extract is attributable to the *Nocardiopsis chiA*-like chitinases detected in the sequenced aliquot of the extract and represents the first example of an active exoenzyme extracted, assayed and sequenced from a soil.

The efficiency of mass spectrometry via in-gel digestion would preclude recovery of low-abundance peptides. *Nocardiopsis*-like proteins
must therefore contribute disproportionately to the functional activity of the soil and thus the degradation of chitin. This is in marked contrast to the prevalence data for 16S rRNA gene analysis and GH18 chi gene analysis. Despite numerous attempts it was not possible to cultivate Nocardiopsis-like strains directly from the soil.

Conflict of Interest
The authors declare no conflict of interest.

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