Bioturbation by bandicoots facilitates seedling growth by altering soil properties

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Abstract

1. Animals that forage for food via bioturbation can alter their habitat, influencing soil turnover, nutrient cycling and seedling recruitment, effectively acting as ecosystem engineers. Many digging mammals forage for food by digging small pits and creating spoil heaps with the discarded soil. We examined how small-scale bioturbation, created by the foraging actions of an ecosystem engineer, can alter soil nutrients and subsequently improve growth of plants.

2. We investigated the microbial and chemical properties of soil disturbed by the foraging of an Australian marsupial bandicoot, quenda (*Isoodon fusciventer*). Soil was collected from the base of 20 recent foraging pits (pit), the associated spoil heaps (spoil) and adjacent undisturbed soil (control) and analysed for nutrients (phosphorus, potassium, sulphur, organic carbon and conductivity) and microbial activity. Soil cores were collected from the same locations and seeds of the dominant canopy species, tuart (*Eucalyptus gomphocephala*), added to the soil under glasshouse conditions. The growth of seedlings were measured (height, maximum growth, basal stem width, shoot and root biomass) over a four-month period and arbuscular mycorrhizae (AM) fungi colonisation rates of seedling roots investigated.

3. Soil from the spoil heaps had the greatest levels of conductivity and potassium. Both the spoil and undisturbed soil had greater amounts of microbial activity and organic carbon. In contrast, the pits had less nutrients and microbial activity.

4. Seedlings grown in spoil soil were taller, heavier, with thicker stems and grew at a faster rate than seedlings in the pit or control soil. Colonisation with AM fungi was greatest for seedlings grown in pit soil. The best predictors of seedling growth were greater amounts of potassium, electrical conductivity and microbial activity. The best predictor of higher colonisation rates of AM fungi was less phosphorus.

4. Bioturbation by ecosystem engineers, like quenda, can alter soil nutrients and microbial activity, facilitating seedling growth. We propose this may be caused by enhanced litter decomposition beneath the discarded spoil heaps. As the majority of Australian digging mammals are threatened,

with many suffering substantial population and range contractions, the loss of these species will have long-term impacts on ecosystem processes.

Key Words: arbuscular mycorrhizal fungi; bandicoot; digging mammals; ecosystem engineer; plant-animal interactions; plant-animal-microbe interactions.

Introduction

Bioturbation by animals that dig, burrow or displace soil while searching for food can influence their environment in many ways (Whitford & Kay 1999). Digging animals alter the physical and chemical properties of soils, modify resource pathways and alter the availability of resources for other species; and; consequently many digging animals are considered ecosystem engineers (Davidson, Detling & Brown 2012; Coggan, Hayward & Gibb 2018). Species that dig when foraging for food create small-scale disturbances that may be quite ephemeral in nature; however, small-scale bioturbation actions may cumulatively impact ecosystems (Darwin 1881). When digging animals are numerous, the foraging pits they create can be plentiful and subsequently influence environmental processes (Alkon 1999; Eldridge *et al.* 2012). Here, we examine how foraging pits created by quenda, *Isoodon fusciventer*, an Australian digging marsupial, may alter soil nutrients and consequently facilitate seedling growth.

By foraging in the soil, animals break through the soil crust, often mixing soil types and horizon layers (Alkon 1999). In arid environments, breaking the soil crust can reduce soil hydrophobicity, while simultaneously allowing moisture to infiltrate the top layer of soil (Garkaklis, Bradley & Wooller 1998; Valentine *et al.* 2017), at least initially. The pit created often acts as a sink for organic matter, trapping sediment, litter and seeds, altering soil fertility at local scales (Garkaklis, Bradley & Wooller 2003; Eldridge & Mensinga 2007; James, Eldridge & Hill 2009; Hagenah & Bennett 2013). When a foraging pit is created, it usually has an associated spoil heap of evacuated soil, also known as ejecta mounds (Whitford & Kay 1999). The combination of digging and discarding soil disrupts the

69 microhabitat layer by exposing soil at the digging site, and burying organic matter and litter under the
70 spoil heap; subsequently altering surface litter composition and potentially contributing to litter
71 decomposition (Valentine *et al.* 2017). The burial of litter is an important component in litter
72 decomposition (Beare *et al.* 1992; Austin, Araujo & Leva 2009) and the digging or raking activities of
73 some animals, such as heteromyid rodents, the short-beaked echidna (*Tachyglossus aculateatus*) and
74 malleefowl (*Leipoa ocellata*) enhance litter decomposition in arid environments (Eldridge *et al.* 2012;
75 Smith, Avitabile & Leonard 2016; Travers & Eldridge 2016).
76
77 The engineering activities of animals that enhance litter decomposition can facilitate a change in soil
78 nutrients (see Platt *et al.* 2016 for review); although these are often inconsistent, varying among
79 organisms, bioturbation type and intensity of digging (Yu *et al.* 2017). For example, the burrows of
80 mole-rats (Bathyergidae) have more nitrogen compared to undisturbed soil (Hagenah & Bennett
81 2013); while mounds of pocket gophers (*Thomomys tadpoides*) are predominantly associated with
82 lower levels of nitrogen (Yurkewycz *et al.* 2014). Foraging pits created by badgers (*Meles meles*)
83 have more potassium (Kurek, Kapusta & Holeksa 2014) as do those made by burrowing bettongs
84 (*Bettongia lesuer*) and greater bilbies (*Macrotis lagotis*) (James, Eldridge & Hill 2009), although there
85 appears to be no effect on potassium or phosphorus levels in foraging pits created by the woylie (*B.*
86 *penicillata*)(Garkaklis, Bradley & Wooller 2003).
87
88 As many nutrients (e.g. nitrogen, phosphorus and potassium) are essential for adequate plant growth,
89 burrowing or foraging that encourages litter decomposition may subsequently lead to enhanced
90 seedling recruitment and/or growth. Greater seedling recruitment was observed in areas with digging
91 marsupials (James, Eldridge & Moseby 2010) and in experiments using artificial diggings compared
92 to undisturbed areas (Valentine *et al.* 2017). Glasshouse trials also indicate that when grass seedlings
93 are grown in the soil of foraging tracks created by echidna, the seedlings grew taller than seedlings
94 grown in undisturbed soil, especially under challenging conditions (Travers *et al.* 2012).
95 Furthermore, the composition of many vegetation communities are considered to be influenced by the

presence (or the removal) of digging animals (Whitford & Kay 1999; Davidson, Detling & Brown 2012).

Digging activities of animals are also linked to changes in soil microbial communities, with foraging activities of echidnas influencing ecosystem function, measured by enzyme concentrations, compared to undisturbed soils (Eldridge *et al.* 2016). Digging mammals can also be key dispersers of mycorrhizal fungi, via consumption of the fruiting bodies and subsequent defecation of viable spores (Johnson 1996; Tay *et al.* 2018). Mycorrhizae are specialised structures arising from the association of plant roots and fungi, that allow plants greater access to limited soil nutrients (e.g. nitrogen and phosphorus) and water (Smith & Smith 2011), with an estimated 72% of vascular plants forming symbiotic associations with arbuscular mycorrhizae (AM) (Brundrett & Tedersoo 2018). Indeed, the presence of small mammals positively influenced AM colonisation of roots in semi-arid Chilean shrubland (Aguilera *et al.* 2016), while in Western Australia woodlands mycorrhizal communities differ in areas with abundant digging mammals (Dundas *et al.* in press).

Many of the world's digging mammals are threatened (Davidson, Detling & Brown 2012). The loss of these ecosystem engineers may lead to a subsequent loss of the ecological processes they perform and important plant-animal interactions. Globally, conservation efforts include reintroductions of threatened species, increasingly not only for the conservation of species themselves, but also in attempts to restore lost ecosystem functions (e.g. Law *et al.* 2017); and greater understanding on the role of digging mammals in ecosystem function is therefore required (Coggan, Hayward & Gibb 2018). Australia has the world's highest record of mammal extinction in the last 200 years (Woinarski, Burbidge & Harrison 2015) and a large proportion of extant digging marsupials are threatened or have suffered severe range contractions (Fleming *et al.* 2014). Many of these species are within the critical weight range category (35–5500 g) and are highly susceptible to predation by introduced red foxes (*Vulpes vulpes*) and feral cats (*Felis catus*), in addition to habitat loss and inappropriate fire regimes (Johnson & Isaac 2009; Woinarski, Burbidge & Harrison 2015).

We examined the role of quenda foraging in facilitating plant growth. Previous research indicates this species is an important ecosystem engineer, with an individual quenda creating ~45 pits each night and displacing nearly four tonnes of soil annually per individual (Valentine *et al.* 2013). The foraging pits of quenda can also reduce soil water repellency, increase soil moisture and reduce litter size within a few months of creation (Valentine *et al.* 2017). In addition, seedling recruitment of co-occurring native tree species (e.g. *Eucalyptus gomphocephala* and *Acacia saligna*) is greater in artificially dug soil compared to undisturbed soil (Valentine *et al.* 2017). Our research further explores the role of quenda in manipulating soil and plant properties by examining: *i*) whether soil nutrients are different between recently created quenda foraging pits (both the foraging pit and associated spoil heap) and undisturbed soil; *ii*) difference in growth of seedlings and AM fungi colonisation of seedling roots grown in quenda-manipulated soil and undisturbed soil and, *iii*) whether soil nutrients and microbial activity can predict seedling growth and AM colonisations.

Methods:

The quenda is a medium-sized (weighing 800–1200 g) omnivorous marsupial that searches for food (e.g. invertebrates, tubers and fungi) by digging foraging pits (Valentine *et al.* 2013). Previously considered a subspecies of the threatened southern brown bandicoot (*Isoodon obesulus*) (Travouillon & Phillips 2018), the quenda has similarly suffered population decline, principally as a result of introduced predators and habitat loss, throughout its range in south-western Australia. The quenda persists in forest remnants and peri-urban reserves where vegetation cover is sufficient to provide protection from predators (Bryant *et al.* 2017), although these small meta-populations are vulnerable to disturbances (Ramalho *et al.* 2018). While digging for food, quenda create conical-shaped foraging pits (~100 mm across and 70 mm deep), with soil ejected from the pit forming a spoil heap (ejecta mound) that covers the undisturbed ground surface and any litter present (Valentine *et al.* 2013).

Yalgorup National Park (32°50′54.52″S; 115°40′08.72″E) within the Swan Coastal Plain bioregion (Thackway & Cresswell 1995) in south-western Australia, supports a naturally-occurring population of quenda. The region has a Mediterranean-type climate with hot, dry summers and mild, wet winters

with average annual rainfall of 864 mm (Bureau of Meteorology, station # 009679). Our work was conducted on the Spearwood Dune system (predominantly yellow-phase Karrakatta sands) where the habitat was open woodland dominated by *Eucalyptus gomphocephala* (tuart), with scattered *E. marginata* (jarrah) and *Corymbia calophylla* (marri) and a mid-storey *Banksia* spp. (for detailed vegetation description, see Valentine *et al.* 2013; Valentine *et al.* 2017). The dominant eucalypt, tuart, has been the focus of restoration trials within sections of Yalgorup National Park (see Ruthrof *et al.* 2016). South-western Australian soils are old, leached and nutrient deficient (McArthur & Bettenay 1960; Henderson & Johnson 2016), and consequently mycorrhizal fungi play an important role in maintaining plant health.

Soil nutrients

We identified 20 recent foraging pits created by quenda, within the previous 1 – 2 months, at Martin's Tank, Yalgorup National Park (29/10/2012). Samples from three locations along the foraging pit profile were collected: *i*) the base of the foraging pit (hereafter called pit), *ii*) the spoil heap or ejecta mound (spoil), and *iii*) adjacent undisturbed ground, located within 0.5 m of the foraging pit (control). From each location, we collected soil samples (~ 150 g) for nutrient analyses. Standard soil nutrient analyses, undertaken by CSBP Soil and Plant Analysis Laboratory (Bibra Lake, Western Australia), examined nutrient quantities that may be important for plant growth: nitrate nitrogen (mg/kg), ammonium nitrogen (mg/kg); phosphorus (mg/kg; Colwell), potassium (mg/kg; Colwell), sulphur (mg/kg; KCI 40), organic carbon (carbon, %; Walkley-Black), as well electrical conductivity (dS/m; which provides an indication of the level of nutrient salts present (Landis 1989) and pH level ($CaCl_2$ and H_20).

Microbial activity

To estimate the overall microbial activity in each sample, we undertook a fluorescein diacetate (FDA) hydrolysis assay, which measures the enzyme activity (including lipases, esterases and proteases) of microbial populations (using methods following: Schnürer & Rosswall 1982; and Adam & Duncan 2001). We collected soil samples (~5 g) from the top 5 cm of soil from the three locations: pit, spoil

and control. Activity of the enzymes results in the hydrolytic cleavage of FDA (colourless) into fluorescein (fluorescent yellow-green). Enzyme activity is quantified by assessing the intensity of colour using spectrophotometry (490 nm). A range of fluorescein dilutions was used (n = 5) to generate a standard curve and optical densities converted to µg fluorescein produced per gram of soil.

Plant growth and colonisation of arbuscular mycorrhizal fungi

To examine growth of seedlings, we collected 60 soil cores from the three locations (pit, spoil, and control from 20 replicate foraging pits) using a cylindrical corer (plastic PVC pipe inner diameter: 27 x 10 cm L x W) and carefully transferred the soil to standard, free-draining pots of similar dimensions to the corer with minimal disturbance of soil. Pots were placed in a glasshouse, seeded with 10 *E. gomphocephala* seeds into each pot (3/11/2012) and watered automatically once daily. Germination was successful with all pots containing seedlings (median 8 seedlings per pot) and were thinned to the largest single seedling per pot (at 7 weeks, 21/12/ 2012). We measured seedling height (cm) every 7–12 days, with a total of 13 measurements over a 3-month period. Prior to harvesting (25/03/2013), we measured the final height and stem width (mm, using digital callipers, 1 cm from the soil surface). Shoots were harvested using secateurs to cut the shoot off at 5 mm from the soil surface and were dried at 70°C for 3 days, before weighing (g).

To collect root material, we gently removed roots from the pots and washed the root mass to remove excess soil, then gently dried with paper towels. Fine roots were identified using visual inspection and a small sample (~0.5 g) was carefully removed into a fine sieve (0.5 mm) to examine AM colonisation. Fine root samples were stored in 70% ethanol, with remaining root material dried at 70°C for 3 days before weighing (g). Fine roots (<1 mm in diameter) were later fixed in formalin acetic acid (FAA) solution (13 ml formalin + 5 ml acetic acid + ethyl alcohol) and cut into 1-cm-long segments. Mycorrhizal colonization was assessed according to methods described by Brundrett et al. (1984). The root segments were washed with water and placed in 20-ml vials containing 10% KOH solution and incubated for 30 min at 90°C. Roots were washed with water and dyed with 0.05% trypan blue solution (lactic acid : glycerol : distilled water = 1 : 2 : 2) and maintained at 50°C

overnight. Ten randomly selected root segments per plant replicate were mounted on each of three microscope slides and examined for mycorrhizal colonisation under an Olympus BX50 transmitted light bright field microscope (Olympus, Japan). The number of colonised root sections was counted and summed across the three slides and converted to a proportion of the 30 root sections examined.

Statistical analyses

Individual seedling trajectories were fitted by modelling seedling height using a Gamma distribution as a smooth function of time since sowing via the gamm4 package (Wood & Scheipl 2013) in R (R Core Team 2016). The resulting smoothed model trajectories were used to calculate rate of maximum growth for each seedling (mm day^{-1}). We used a hierarchical mixed modelling approach to examine the strength of the effect of the foraging pit location (pit, spoil, control) on both soil characteristics (conductivity, nutrients and FDA) as well as the plant growth response variables. The variables final height, max growth, dry shoot biomass, dry root biomass and stem width were modelled using a gamma distribution, and the proportion of AM in roots were modelled using a binomial distribution based on the 30 observations. As soil characteristic variables were used as predictors of the plant growth response variables in subsequent analyses (see below) they were transformed (where necessary) to optimize spread across the predictor range and improve scaling relationships. The nutrients phosphorus, potassium and sulphur were natural log transformed, FDA was cube-root (cbrt) transformed and conductivity was square-root (sqrt) transformed. Following transformations all soil characteristic variables were modelled via a Gaussian distribution. Each variable was modeled using a Generalized Linear Mixed Model (GLMM), including foraging pit identifier as a random intercept to account for non-independence of the three locations (pit, spoil and control) sampled at each foraging pit replicate. Initial models were fit using the function glmer from the lme4 library (Bates *et al.* 2015) in R, with resulting model output used to calculate AICc and a pseudo R^2. Equivalent models were fit in a Bayesian context based on uninformative priors using the INLA package (Lindgren & Rue 2015) in R and the inla.posterior.sample used to generate 95% credible bounds for model parameters that were used to interpret significant differences among locations. Two models were fitted for each variable: the null model including only an intercept and the foraging pit identifier and a model

including foraging pit location (pit, spoil, control). Differences in the AICc and pseudo R^2 values between the location and null models were used to evaluate the strength of the effect of foraging pit location for each variable.

Differences in the growth trajectories of seedlings among the three foraging pit profile locations were assessed using Generalized Additive Mixed Models (GAMMs) based on a Gamma distribution with a log link function, with foraging pit identifier included as a random intercept term as in the GLMM above, but an additional seedling identifier random intercept to account for repeated measurement on individual seedlings over time.

We explored the relative importance of the relationship among the soil characteristics [phosphorus (mg/kg), potassium (mg/kg), sulphur (mg/kg), carbon (%), pH ($CaCl_2$), electrical conductivity (dS/m) and FDA (µg hydrolysed FDA / g of dry soil)] as predictors of the plant growth variables [response variables: final height (mm); stem width (mm); maximum growth (cm/day); dry shoot biomass (g); dry root biomass (g); AM colonisation (%)] using a full sub-sets GAMM approach via the function full.subsets.gam described in Fisher *et al*. (2018) in R using the default argument settings, with the exception that maximum model size was limited to two simultaneous predictors. This approach constructs a complete model set excluding any models containing correlated > 0.28 Pearson correlation and compares these using Akaike Information Criterion (AICc), Bayesian Information Criterion (BIC) and AIC weight (ωi) values (Burnham & Anderson 2002). The simplest model within 2 AICc of the model with the lowest AICc was assumed to be the optimal model, with the relative importance of each predictor across the whole model set calculated as summed model weights. All models were fit using GAMMs, via the gamm4 function from the gamm4 package (Wood & Scheipl 2013) in R using the appropriate statistical distribution and random structure as described for the GLMs above.

Results

Soil nutrients

Many of the soil physiochemical properties (e.g. conductivity, Fig. 1a and potassium, Fig. 1c) were significantly greater in the spoil soil than either the pit or control soil. Carbon was least in the pit compared to either spoil or control soil (Fig. 1d). Soil location significantly influenced conductivity, potassium and carbon, with models including foraging pit location having substantially smaller AICc values than the null models (Fig. 1). Although there was a trend for higher levels of phosphorus and sulphur in the spoil compared to pit soil (based on 95% CI; Fig, 1b and 1e), the AICc, models including location had very little support, indicating that differences were not strong. The pH level (both $CaCl_2$ and H_20) did not vary among the foraging pit locations, and is not considered in any further analyses (not shown on Fig. 1).

Soil microbial activity

There was more microbial activity in the spoil and control soil, indicating the pit soil was comparatively sterile, and soil microbial activity, indicated by hydrolysed FDA (Fig. 1f), showed strong support for the inclusion of soil location in a model.

Plant growth and colonisation of arbuscular mycorrhizal fungi

There was no difference in the number of seedlings that germinated among foraging pit locations at seven weeks post-sowing in pots in the glasshouse trial (location mean seedlings ±95% CI; Pit = 7.8 ± 1.1; Spoil = 7.8 ± 0.7; Control = 7.5 ± 0.8). Location along the foraging pit had a strong influence on seedling growth over time with seedlings grown in the spoil soil taller than seedlings grown in pit or control soil (location model AICc: 2778.3 versus null model AICc: 2815.3; Fig. 2). Seedlings in the spoil soil were already slightly taller than seedlings in the pit soil at the first measurement (49 days since sowing) and by the third measurement (62 days since sowing) differences in the heights of seedlings among foraging pit locations were distinct (Fig. 2). Seedlings grown in the spoil soil grew more rapidly than seedlings grown in either the pit (2.8 times faster) or control (~2 times faster) soils (Fig. 3b), especially in the first 40 days of measurements (Fig. 2). At the time of harvest (142 days since seeding), seedlings from the spoil soil were double the height of pit seedlings and 1.5 times taller than the control seedlings (Fig. 3a). At harvest, seedlings grown in the spoil soil had the greatest

shoot biomass (4 times heavier than seedlings from the pit; Fig 3c), stem width (Fig. 3d) and root biomass (3.5 times heavier than seedlings from the pit; Fig. 3e). By contrast, seedlings grown in the pit soil were consistently the shortest seedlings (Fig. 2 & 3). Seedlings in the pit soil had the narrowest stems (Fig. 3d) and smallest shoot biomass (Fig. 3c), while their root biomass was not different to the seedlings grown in the control soil (Fig. 3e). The seedlings grown in the pit soil, despite being typically the smallest seedlings observed, exhibited the greatest proportion of AM colonization (4 times greater than for seedlings from the pit; Fig. 3f).

Predictors of seedling growth

Potassium, phosphorus, FDA and electrical conductivity were the strongest predictor variables for the six seedlings response variables examined (Fig. 4). For each seedling response variable there was only one preferred model (all other models had ΔAICc > 2), with each model for the seedling response variable containing two predictor variables (Table 1). Seedling final height, stem width and root biomass were positively correlated with the amount of potassium in soil samples. Maximum growth per day and shoot biomass of seedlings was positively correlated with the amount of electrical conductivity of the soil (Table 1; Fig. 4; Figure S1 in Supporting Information). The percentage colonisation of AM was negatively influenced by the amount of phosphorus in the soil (Table 1; Fig. 4; Fig. S1). All seedling response variables were correlated with FDA readings, with bigger seedlings tending to have greater levels of FDA (Table 1; Fig. 4; Fig S1).

Discussion

We have demonstrated that foraging activities of quenda alter soil properties, including nutrient concentrations and microbial activity, which facilitates greater plant growth of young seedlings. The differences in soil properties were most evident in spoil soils (the soil ejected from the foraging pits) where the subsequent growth of seedlings was 1.5–2 times greater than seedlings grown in control (undisturbed) or foraging pit soils. Seedling growth response variables were best predicted by greater amounts of microbial activity and some soil nutrients (potassium and electrical conductivity), which were often greatest in the spoil soils. While it has been demonstrated that quenda foraging alters the

heterogeneity of soil properties at micro-scales, such as increasing water infiltration and decreasing hydrophobicity (Valentine *et al.* 2017), our current results illustrate that digging activities of quenda also significantly increases native plant growth.

Soil disturbance by animals that dig or burrow can have a great impact on soil chemical properties, (Platt *et al.* 2016; Yu *et al.* 2017), with most studies comparing soil from burrows or mounds with nearby undisturbed soil (Coggan, Hayward & Gibb 2018). Far fewer studies examine the more ephemeral foraging pits, although in Australia there has been some research in this field (e.g. Garkaklis, Bradley & Wooller 2003; James, Eldridge & Hill 2009; Travers *et al.* 2012). Our study is the first to compare soil nutrients at different locations along the foraging pit profile and our findings clearly show that digging animals are creating significant nutrient patchiness at a micro-site scale.

Our study demonstrated that the spoil soil of quenda foraging had greater levels of electrical conductivity and potassium than either the undisturbed soil or the pit themselves. Similarly, the burrow spoils of wedge-tailed shearwaters (*Puffinus pacificus*) were greater in electrical conductivity than soil from the bird colonies or surrounding undisturbed vegetation mounds (Bancroft, Garkaklis & Roberts 2005). Although not an active dig, hip holes created by Australian kangaroos (*Macropus* spp.) while they rest, have greater electrical conductivity, which decreases with distance from the hip hole (Eldridge & Rath 2002). Very little is known about how electrical conductivity affects tree species, although seedlings seem sensitive to small changes (Allen, Chambers & Stine 1994). Growth of container-grown seedlings of *Pinus resinosa* from north-eastern North America was greatest at electrical conductivity between 1.8-2.2 dS/m; followed by toxicity at 2.5 dS/m (Timmer & Parton 1984). The ancient, low-nutrient soil of south-western Australia typically has low levels of electrical conductivity (Henderson & Johnson 2016), and small changes in these amounts, such as those observed in this paper (e.g. control soil = 0.16 dS/m *c.f.* spoil soil = 0.22 dS/m), may facilitate seedling growth in the early establishment phase.

In our study, potassium was one of the best predictors of seedling growth. Changes in soil potassium levels have been observed in the burrows created by many digging animals (see Platt *et al.* 2016 for review), although there is little consistency in the direction of change. For example, burrows created by badgers and foxes had more potassium (Kurek, Kapusta & Holeksa 2014) whilst mounds of pocket gophers (*T. talpoides*) had less potassium than undisturbed surface soil (Mielke 1977). In contrast, potassium quantities at foraging pits created by woylies were similar to undisturbed soil (Garkaklis, Bradley & Wooller 2003), and the intensity of digging by plateau pika (*Ochotona curzoniae*) did not modify potassium levels (Yu *et al.* 2017). Potassium plays a key role in plant growth and development through the movement of water, nutrients and carbohydrates in plant tissue (Marschner 1995). Potassium is considered to be an essential nutrient that can significantly ameliorate plant abiotic stress (Marschner 1995); and previous studies have associated greater levels of potassium with increases in tree growth, wood production, leaf gas exchange, stomatal sensitivity to water deficit, and water use efficiency (Battie-Laclau *et al.* 2016). The addition of potassium to soil can also result in a positive effect on the growth of tropical forest seedlings (Santiago *et al.* 2011), while potassium deficiency can result in reduced plant growth (Marschner 1995). Our research is one of the first studies that demonstrate a clear relationship between animal foraging activities, changes in nutrient levels and subsequent plant growth. Given that potassium is highly mobile and readily leached in soils, for the nutrient deficient soils of south-western Australia (McArthur & Bettenay 1960; Henderson & Johnson 2016), even small increases in potassium (such as those created in the wake of quenda digging activities) could make a difference to early seedling development.

Microbial activity (FDA) was significantly less in pit soil than for spoil and control soil. The depth of the foraging pit may contribute towards this observation, as microbial activity declines with increasing soil depth (Taylor *et al.* 2002). Quenda digs at Yalgorup National Park have a depth of ~ 70 mm (range 35 - 135 mm; Valentine *et al.* 2013). Consequently, the bottom of the pit (where pit soil was sampled) may have been below the level of high microbial activity. Previous research has indicated that burrowing by invertebrates (e.g. earthworms, Aira *et al.* 2010) can also increase FDA levels, but, this is the first study to show an increase in FDA through the digging actions of mammals.

372 Further research examining the influence of digging on the composition and function of microbial
373 communities and how they impact seedling germination and establishment, would be valuable.
374
375 Mycorrhizal mutualisms are particularly important for plant growth; specifically, these fungi increase
376 the ability of plants to take up phosphorus, nitrogen and micronutrients, and are a defence against
377 plant pathogens (see review by Smith & Smith 2011). We found that AM colonisation was greater in
378 seedlings grown in the pit soil, and that low levels of soil phosphorus were a predictor of high AM
379 colonisation. Phosphorus is an important nutrient for plant growth, but can be challenging for plants to
380 take up; AM fungi-plant mutualisms are an effective pathway for plants to acquire phosphorus, which
381 can assist in root growth (Smith *et al.* 2011). The seedlings grown in the pit soil had ~one third of the
382 root biomass compared to seedlings grown in the spoil heap, and it is possible that this greater root
383 biomass reduced the ratio of AM root colonisation to non-colonisation (Smith & Smith 2011). In
384 addition, it is unclear how the differences in AM colonisation of roots would affect seedling growth
385 over longer time frames.
386
387 *Why does quenda digging facilitate seedling growth?*
388 In our study, seedling growth was substantially greater for seedlings grown in soil from the spoil heap
389 created by quenda than either the foraging pit or adjacent undisturbed soil. Potential reasons for
390 enhanced seedling growth may be due to reduced bulk density of soil in the spoil heaps and altered
391 litter decomposition rates. Although we did not measure bulk density among the soil treatments,
392 previous research has identified that spoil heaps created by digging animals often have lower bulk
393 density than undisturbed soils (reviewed in Platt *et al.* 2016). In manipulative experiments, lower soil
394 bulk density positively affected many growth parameters of Scots pine (*Pinus sylvestris*) and
395 European beech (*Fagus sylvatica*) seedlings (Kormanek, Banach & Sowa 2015). In addition to the
396 potential changes in soil density, we propose that foraging by quenda created an environment
397 conducive for litter decomposition in the spoil heap that subsequently returned nutrients to the soil,
398 making them available for plant up-take and therefore facilitating seedling growth. The facilitation of
399 seedling growth by animal digging activities has previously been demonstrated with Australian grass

seedlings (*Dactyloctenium radulans*) grown in 18-month old echidna foraging pits and undisturbed surface soil in a glasshouse experiment (Travers *et al.* 2012). Seedlings grown in the echidna foraging pit soils had greater biomass, greater proportional reproductive effort and growth rate than those growing on surface soils.

Litter decomposition is a major determinant of nutrient cycles for many terrestrial ecosystems, with decomposition returning nutrients (including potassium) captured in plant material to the soil (Aerts 1997). Decomposition rates are influenced by climate, litter chemistry (Aerts 1997), soil microbial and fungal communities (Beare *et al.* 1992) as well as litter position (above vs below ground) and microhabitat characteristics (Austin, Araujo & Leva 2009). Buried litter decomposes faster than surface litter (Austin, Araujo & Leva 2009), potentially due to the increased exposure to microbial and fungal communities (Beare *et al.* 1992), with microbial communities varying in association with animal foraging activities (Eldridge *et al.* 2016).

Even though we collected the soil, and sampled the nutrients, when the foraging pits were still relatively fresh (within ~2 months of creation), the greater levels in the spoil soil we observed is likely to have occurred due to greater rates of litter decomposition (with the spoil heap containing surface litter that was buried by the spoil heap). During the glasshouse trial, the litter in the spoil soil may have continued to decompose (especially given the constant supply of water), adding nutrients to the soil, and potentially accounting for the relatively steep growth rate of seedlings grown in the spoil heaps within 2 – 3 months since seeding (Fig. 1). In contrast, the pit had captured very little litter and had low levels of microbial activity potentially explaining the slow seedling development. In the field, we have observed the spoil heap partially degraded into the pit, and the foraging pits of digging mammals often becomes a reservoir that collects litter (and seeds) over time (James, Eldridge & Moseby 2010). The combination of increased nutrients, reduced soil bulk density and greater water infiltration (Valentine *et al.* 2017) provide important sites for seedling germination, establishment and growth.

Conclusions

The micro-scale disturbances created by digging mammals may be incredibly important for ecosystem functioning, facilitating changes in soil nutrients, microbial activity and plant growth. Our study clearly shows that foraging by quenda can alter soil nutrient and microbial activity that subsequently influences plant growth. Of concern in Australian ecosystems, is that the vast majority of digging mammals are threatened (Fleming *et al.* 2014) and many landscapes no longer contain these ecosystem engineers, or if they do, the animals are in substantially reduced numbers. The loss of digging mammals goes hand-in-hand with the loss of their functional role in maintaining landscapes. Consequently, our understanding of the biotic and abiotic ecological interactions of Australian landscapes may be impoverished by not accounting for their presence. Further research is needed to understand the role of digging mammals in landscapes, as well as whether the return of such species may aid, or hinder, landscape restoration processes.

Authors' Contributions

LV and KR conceived the ideas and designed methodology; LV, KR and RF collected the data; LV and RF analysed the data; LV and KR led the writing of the manuscript with contributions from GH, RH, RF and PF. All authors contributed critically to the drafts and gave final approval for publication.

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Data Accessibility

Raw data used in this research article are accessible via the University of Western Australia Research Repository: https://doi.org/10.4225/23/5b16364fb037e.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Figure S1. GAMM fits for the top-ranking models for seedling response variables with predictor variables for seedlings grown in soil collected from different locations of a quenda (*Isoodon fusciventer*) foraging pit.

Figures

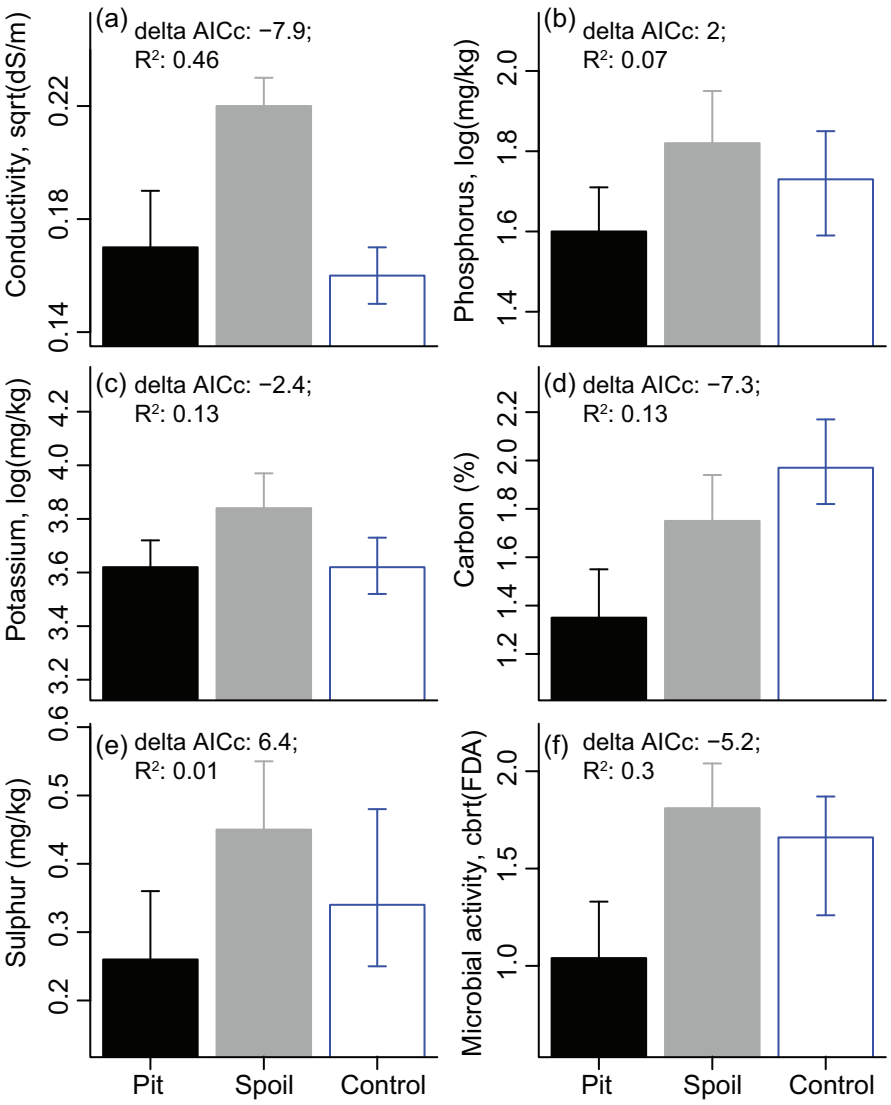
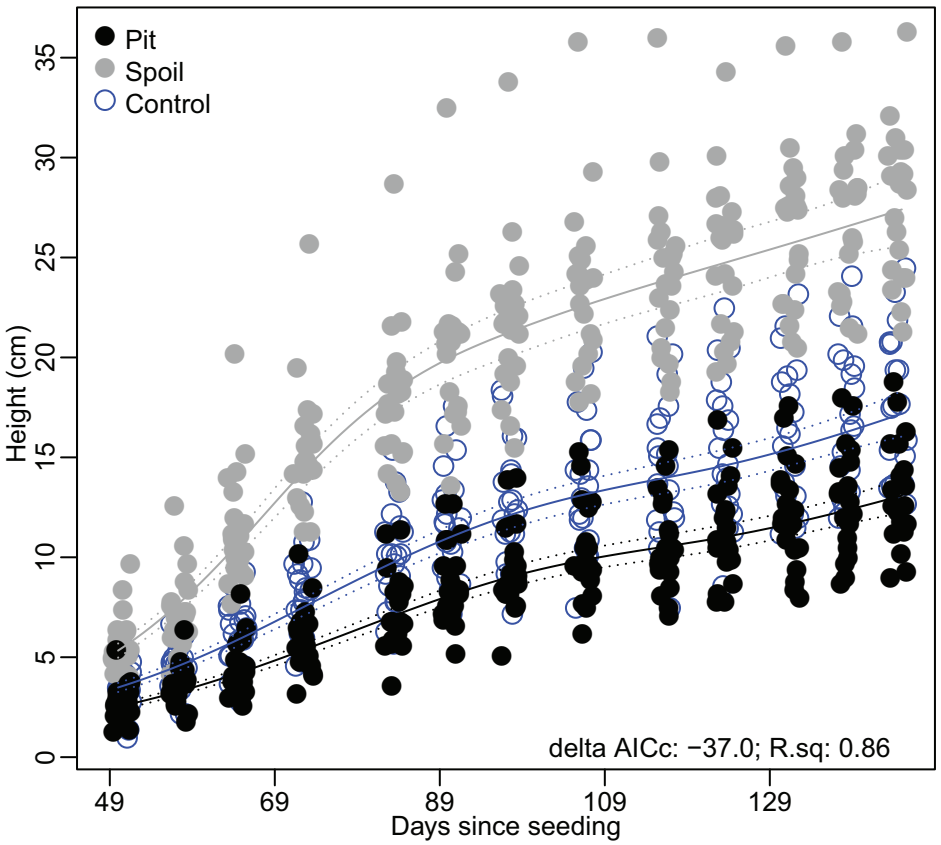


Fig 1. Conductivity (dS/m), nutrient levels (mg/kg) and microbial activity (FDA) of soil collected from different locations of a foraging pit created by quenda (*Isoodon fusciventer*). Means (±95% Credible Intervals, based on estimated Bayesian posterior parameter estimates) are plotted. Delta AICc and R.sq values show the difference in AICc and R^2 values between GLMMs based on location and null models respectively (e.g. location model AICc – null model AICc). Negative delta AICc values indicate that the model containing location was a better fit than the null model (i.e. the AICc value of the location model was less than the AICc of the null model).



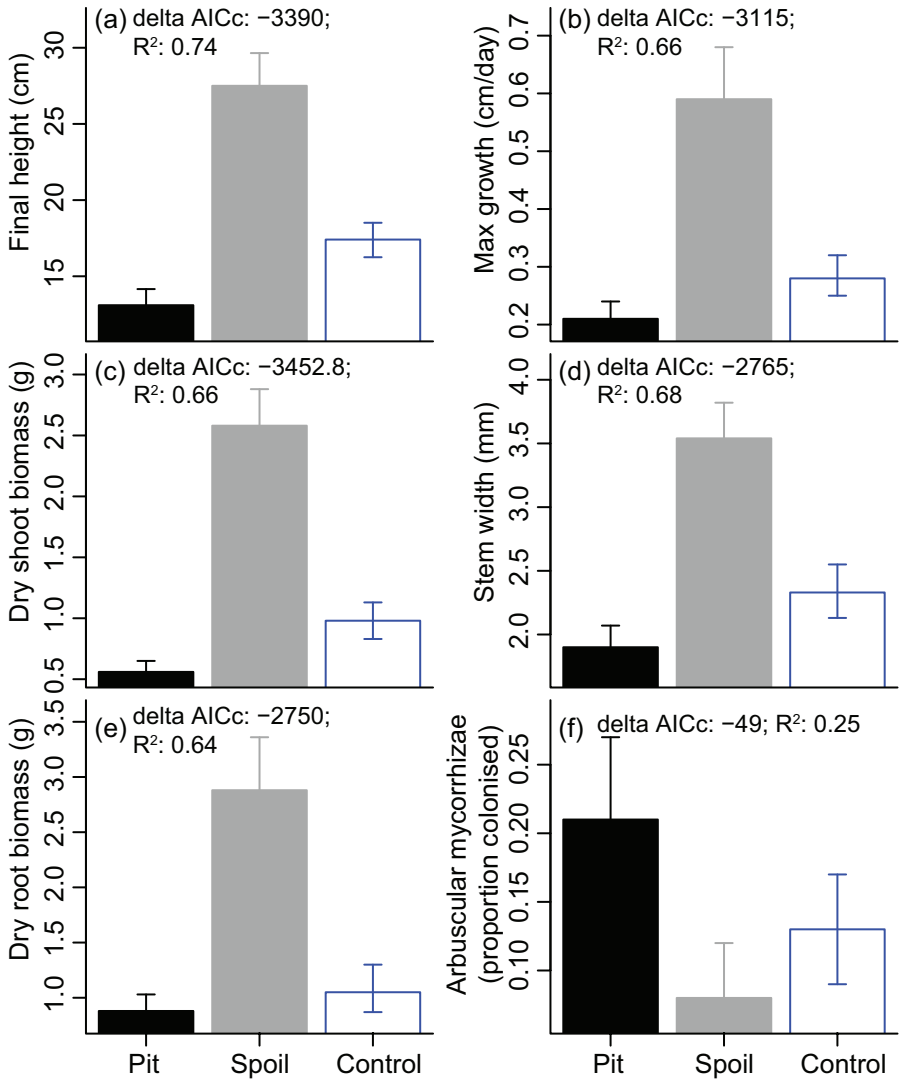
641

642 Fig. 2. Growth of seedlings over time grown in soil collected from different locations of a foraging pit

643 (pit, spoil and control) created by quenda (*Isoodon fusciventer*). Solid lines show GAMM fits for

644 each treatment, dotted lines indicate estimated 95% Confidence Limits.

645



646

647 Fig. 3. Seedling response variables and proportion of arbuscular mycorrhizal (AM) fungi colonisation
648 on roots from seedlings grown in soil collected from different locations of a foraging pit created by
649 quenda (*Isoodon fusciventer*). Plotted are means (±95% Credible Intervals, based on estimated
650 Bayesian posterior parameter estimates). Delta AICc and R.sq values show the difference in AICc
651 and R^2 values between GLMMs based on location and null models respectively (e.g. location model
652 AICc – null model AICc). Negative delta AICc values indicate that the model containing location was
653 a better fit than the null model (i.e. the AICc value of the location model was less than the AICc of the
654 null model).

655

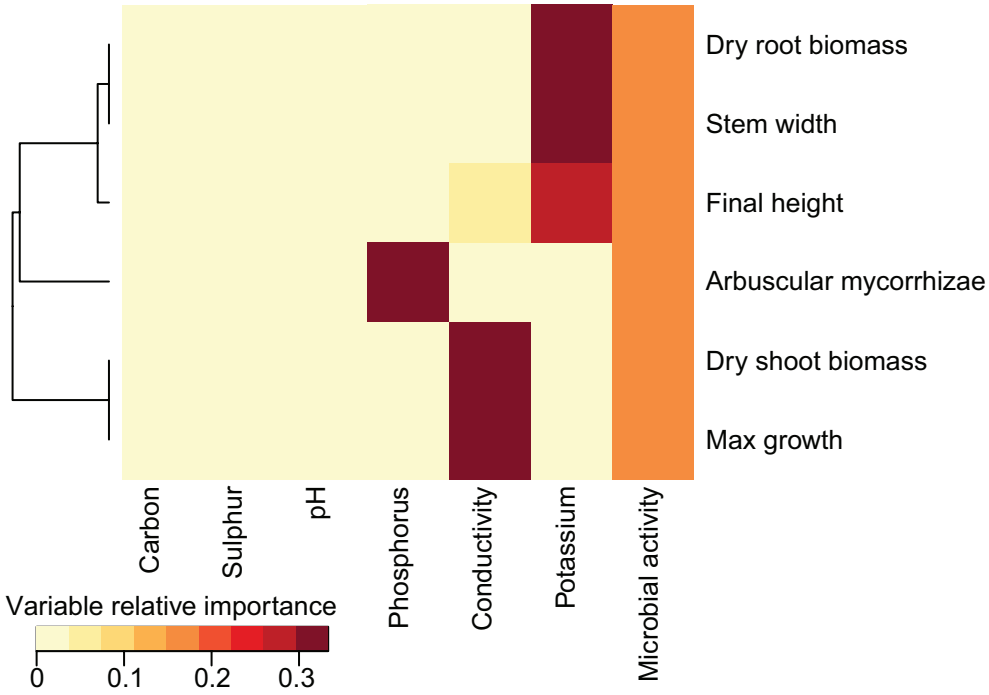


Fig. 4. Heat-map indicating the relative importance (summed AICc weights/number of models) of each predictor variable (FDA, potassium, electrical conductivity, phosphorus, pH, sulphur and carbon) in contributing towards each seedling response variables (maximum growth, shoot biomass, AM colonisation, final height, stem width and root biomass) of seedlings grown in soil from different locations of a foraging pit created by quenda (*Isoodon fusciventer*). Dendrogram shows a Euclidian hierarchical cluster analysis (complete linkage) of the seedling response variables based on the relative importance of the different predictor variables.

665 **Tables**

666 Table 1. Top-ranking generalised additive mixed models (GAMM) for seedling response variables
667 with soil nutrients and microbial activity predictor variables from seedlings grown in soil collected
668 from different locations along the foraging pit profile created by the quenda (*Isoodon fusciventer*).
669 Models included are the top-ranking model (i.e. ΔAICc = 0) for each response variable.

Response variable	Model	df	Adjusted-R^2	AICc weight
Final height (mm)	Potassium + microbial activity	8.74	0.26	0.88
Max. growth (mm/day)	Conductivity + microbial activity	8.38	0.41	1
Shoot biomass (g)	Conductivity + microbial activity	8.07	0.24	1
Stem width (mm)	Potassium + microbial activity	8.45	0.19	1
Root biomass (g)	Potassium + microbial activity	8.07	0.15	1
Arbuscular mycorrhizae (proportion colonised)	Phosphorus + microbial activity	4.58	0.18	0.92

670

671