

Live reports from the soil grain – the promise and challenge of microbiosensors

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Summary

1. Linking microbial activity with ecosystem function is a continuing goal among ecologists focusing their efforts below ground in terrestrial ecosystems.
2. Genomic approaches, using DNA and RNA extracted from soil to characterize types of microbes present and genes expressed in soil, are promising, but, the required destructive harvest confounds spatial and temporal information.
3. Microbiosensors offer a gene-based way to examine microbial perception of, and response to, the soil environment non-destructively, with high spatial and temporal resolution.
4. In this mini-review, we explore the promise, challenges and tradeoffs associated with the design and deployment of microbiosensors in soil, as well as the interpretation of information derived from them ‘live from the soil grain’. Both the promises and challenges are caused by the facts that: microbiosensors are living organisms with specific traits; they come from a phylogenetically diverse but restricted subsample of enormous soil microbial diversity; they are responsive to internal and external influences on multiple cellular processes (not just promoter induction); and they become part of food chains in non-sterile soils. We examine each of these characteristics, and associated blessings and curses, in the context of using microbiosensors to explore microbial soil ecology.

Key-words: GFP, lux, reporter gene, soil microbial ecology

Introduction

Soil biogeochemical functions, many of which are driven in large part by microbes, are a major focus of ecosystems research today. Community genomic approaches seeking to link microbial community structure and ecosystem function are developing rapidly, and should contribute to understanding of taxonomic and functional biodiversity belowground (Cardon & Gage 2006). However, techniques currently used to extract DNA and RNA from soil destroy small-scale spatial and temporal information, and that spatial and temporal micro-heterogeneity in soils is increasingly suspected to be a dominant influence on microbial distribution and biogeochemical function (e.g. Young & Ritz 1998; Schimel & Bennett 2004; Crawford *et al.* 2005). A complementary approach, capable of non-destructively imparting an *in situ* microbial perspective – ‘live, from the soil grain’ – is offered by genetically engineered microbiosensors.

Like many sensors (living or non-living) arrayed in a focal environment, microbiosensors can provide spatial information about local resource availability or environmental conditions over time. Individual microbiosensors can operate at

µm and mm scales in intact, non-sterile soil, scales that previously have not been readily open to empirical investigation. Several recent reviews have considered uses, strengths and limitations of microbiosensors, from multiple perspectives (Daunert *et al.* 2000; Hansen & Sørensen 2001; Leveau & Lindow 2002; Cardon & Gage 2006). Building on this discussion, we first note several interesting ideas and questions in ecosystems ecology to which microbiosensors can likely contribute useful information, followed by very brief snapshots of information about the soil environment already gleaned using developed sensors. We then step through a series of microbiosensor traits that can be viewed simultaneously as strengths and weaknesses of using microbiosensors to explore soil ecology. Though these living sensors provide a unique way to explore the microenvironments they inhabit, there are trade-offs and limitations to be considered when designing them, when drafting questions that they can help answer, and when interpreting the resulting data.

How might microbiosensors contribute toward understanding ecosystem function?

Answering this question requires a description of what microbiosensors are. Briefly, microbiosensors are living microbes

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(usually bacteria) that have been genetically engineered to express reporter genes either continuously (constitutively) or in response to a stimulus of interest. Expressed reporter genes code for any one of a number of proteins (described in more detail below), some providing visible signals *in situ* that can be imaged continuously at low or high spatial resolution, depending on whether individual bacteria's brightness or the brightness of populations of bacteria in soil are of interest. The bacteria to be engineered can be well-characterized lab strains, or they can also be bacteria isolated from the soil of interest, engineered with a plasmid, and then returned to soil. As we discuss further later, the bacteria must be genetically tractable and culturable, narrowing the field of potential native soil bacteria considerably. But, still, candidate species for isolation and engineering could include e.g. native *Pseudomonas*, *Acinetobacter*, and *Bacillus* species known to be metabolically quite versatile.

Do genetically engineered bacteria behave exactly as their native microbial brethren do in soil? Not necessarily, but they are not necessarily meant to. They are meant to be sensors of an appropriate physical scale to be introduced into the soil environment to explore ideas and mechanistic hypotheses posed at that scale. A short, current list of microbiosensor studies that could contribute to current questions about ecosystem function might include:

- Soil hotspots (microsites) supporting high mineralization rates, potentially with high ammonium or perhaps even nitrate availability, are hypothesized to be important for availability of nutrients to plant roots (Schimel & Bennett 2004). Detecting and imaging such hotspots through time at mm (or μm) root-appropriate scales, using e.g. already-developed nitrate-sensitive microbiosensors (DeAngelis *et al.* 2005), would enable comparisons of the dynamics of appearance and disappearance of such hotspots *in situ* with known dynamics of upregulation of nutrient uptake by roots and/or observed root extension.
- Arbuscular mycorrhizas are thought to be much less capable of producing exoenzymes necessary for decomposition of organic compounds (and thus saprotrophy) than ecto- or ericoid mycorrhizas (Read & Perez-Moreno 2003; Cardon & Gage 2006). However, whether AM hyphae in soil are capable of stimulating the activities of heterotrophic or saprotrophic bacteria directly around them in the soil matrix, either through signalling or provision of resources, remains unclear (reviewed by Jones, Hodge & Kuzyakov 2004). Imaging reporter gene expression from sugar- or energy-sensitive microbiosensors established in soil (e.g. Jaeger *et al.* 1999; Bringham, Cardon & Gage 2001; Herron 2007) during and after AM hyphae extension into the soil volume, would provide an *in situ*, non-destructive testbed for the idea that hyphae may stimulate bacterial activity around them via provision of carbon-based resources.
- Temporal patterns of carbon allocation from shoot to root are well-known for many plant species in agricultural and silvicultural literatures (e.g. Cardon *et al.* 2002), and modelling even of large-scale ecosystem fluxes such as

respiration can be noticeably improved with the inclusion of such plant phenology. Do those allocation patterns necessarily translate directly into parallel temporal patterns of carbon 'loss' from roots to rhizosphere soil (Herron, 2007)? Or can plants uncouple the two fluxes in time? Again, carbon- or energy-sensitive microbiosensors established directly around plant roots in the rhizosphere (e.g. Bringham *et al.* 2001; Herron 2007) enable testing of this idea *in situ*, without destructive harvest, along with explorations of whether that coupling can shift as, for example, plants experience a glut of CO_2 under high CO_2 conditions.

Though the development of microbiosensors has been a challenging road linking microbiology, ecology, and environmental engineering, there are now well-characterized and well-tested microbiosensors that can be more widely deployed to answer ecological questions, guided by mechanistic or mathematical hypotheses like those listed above. Below, we briefly note a handful of examples where microbiosensors have already provided insight into patterns of resource availability in soil, plant-microbe interactions, and microbe-microbe interactions. Then, we discuss the dual nature of microbiosensors – they are used as sensors at microscales, and they are living organisms, leading to a number of paired strengths and weaknesses that we explore in sequence.

What kinds of information have microbiosensors already provided?

As noted above, microbiosensors express reporter genes either constitutively or in response to a stimulus. In the latter case, expression of the reporter gene is commonly under the control of a promoter known to respond to a specific resource or condition of interest in soil. Examples where such microbiosensors have provided insight into plant/microbe interactions, insight that would have been very hard to gain with other commonly used research techniques, include:

- A microbiosensor induced by nitrate availability (DeAngelis *et al.* 2005) detected lower levels of nitrate in the rhizosphere of oat roots, compared with nearby bulk soil, presumably reflecting root uptake of nitrate. That proven ability to detect differences in nitrate concentration at micro-scales bodes well for the potential to detect variations in microsite nitrate availability discussed in the previous section.
- Two microbiosensors, induced by sucrose and tryptophan availability, respectively, illustrated that sucrose was released predominantly from near the tips of oat roots, whereas tryptophan was released predominantly from older root tissue (Jaeger *et al.* 1999), providing insight into the potential for the bathing of resident soil microbial populations with a sequence of nutritionally very distinct compounds when roots extend through bulk soil.
- A microbiosensor induced by galactosides (Bringham *et al.* 2001) showed that alfalfa roots secrete galactosides in a patchy fashion, especially around sites of lateral root initiation, but not at root tips. The concentrations of

secreted galactosides fell off very sharply with radial distance into soil from the root axis, because of diffusion limitations, consumption by microbes, or both.

- A microbiosensor induced by low water potential and originally designed for use on leaf surfaces (Axtell & Beattie 2002) documented the spatial patterns of soil drying correlated with differential water uptake by mature and immature zones of corn roots (Herron 2007). Empirical measurements of soil water potential at such small scales have previously been impossible, though for decades there have been single-root models describing dynamics and spatial distributions of water potentials at mm scales around plant roots (e.g. Hillel 1998). Such models can now be tested against empirical data.
- Finally, a microbiosensor sensitive to N-acyl homoserine lactones (AHLs) known to control quorum sensing indicated that AHLs were increased in rhizosphere soil relative to bulk soil (DeAngelis, Firestone & Lindow 2007), suggesting that induction of some rhizosphere processes (e.g. production of exoenzymes) may be linked to build up of AHL-producing bacterial populations or communities.

A number of microbiosensors have also been constructed to detect pollutants such as VOCs (volatile organic compounds) and heavy metals. These are thoroughly reviewed in Hansen & Sørensen 2001 and Daunert *et al.* 2000, and more examples are provided in Cardon & Gage 2006.

Upon induction by an environmental resource or condition of interest, the selected promoter drives transcription of the reporter gene, producing a protein that is detectable by one of a number of methods. Some reporter proteins are fluorescent, including green fluorescence protein (GFP) and red fluorescence protein (RFP), enabling *in situ* detection in soil by epifluorescence microscopy. Other reporter genes, e.g. the bacterial *lux* genes, produce proteins that can generate bioluminescence (Rattray *et al.* 1995; Lewenza *et al.* 2005). Light produced *in situ* by induced *lux*-containing reporters can be detected non-destructively using a lens (microscope or standard 35-mm camera lens) connected to a sensitive CCD camera. Other reporter genes code for proteins that require destructive harvest for their assay, e.g. the β -galactosidase (*lacZ*) genes and ice nucleation (*inaZ*) genes. The drawback of destructive harvest required by these latter two reporter genes is often outweighed by the well-known reliability of the reporter system (*lacZ*), or the built-in amplification of detectable ice nucleating activity associated with *inaZ* expression. These and other reporter genes and proteins have been thoroughly reviewed elsewhere (Daunert *et al.* 2000; Hansen & Sørensen 2001; Leveau & Lindow 2002; Cardon & Gage 2006).

Paired promise and challenge in living microbiosensors

MICROBIOSENSORS ARE ALIVE

The very traits that make microbiosensors exciting tools for exploring soil environments are traits that also challenge

researchers as they interpret information provided by the biosensors. First and foremost, microbiosensors are not electrical sensors; they are living microbes, reporting on environmental conditions or resource availabilities that are relevant to them. Relevance, however, varies organism to organism, and in the extreme, one microbe's substrate could be another microbe's toxin. As Leveau & Lindow (2002) point out, the production of reporter output (protein, light, etc.) depends not only on the presence of the stimulus of interest but also on the availability of energy, nutrients and appropriate conditions for the specific microbiosensors to operate. If those resources and conditions are not present in the environmental sites hosting microbiosensors, those sites are not 'permissive', and no report molecule will be produced, even if inducing stimuli are present. This is a large problem when the microbial microenvironment is heterogeneous, and poorly understood, as in soil. The problem might be at least minimized by preferentially deploying microbiosensors under conditions where, based on our best knowledge of soil characteristics, they could thrive, e.g. in the rhizosphere where carbon is not limiting. One way to partially alleviate the problem of knowing if an microenvironment can support activity of biosensors is by deploying them in pairs in which one biosensor constitutively expresses a reporter and the other, co-inoculated biosensor contains an inducible reporter that indicates the microbial perception of the resource availabilities, environmental conditions, or physiological states of interest (Ramos, Molbak & Molin 2000; Boldt *et al.* 2004; DeAngelis *et al.* 2005). Expression of both constitutive and inducible genes in a particular location indicates that bacteria are active and have seen inducer. Expression of the constitutive reporter and no expression from the inducible reporter in a particular location indicate that conditions for expression are generally permissive, but signal for the inducible reporter is absent. In a more sophisticated implementation of this idea, single microbiosensors strains could be engineered to doubly report on bacterial activity and the signal of interest using constitutive and inducible reporter genes that can be independently and simultaneously monitored. Such a system was used by DeAngelis *et al.* (2005) to investigate nitrate availability in the rhizosphere.

MICROBIOSENSORS NEED TO BE CULTURABLE AND GENETICALLY TRACTABLE

Another essential characteristic for a microbial strain to be used as a microbiosensor is that the strain be culturable, genetically tractable and able to thrive under the conditions of interest. There are many microbes that do not fit this requirement, and among them are some that are of particular interest to those striving to link microbial activity with ecosystem function. For example, ammonia oxidation in soil is catalyzed by AmoA in bacteria from genetically intractable *Nitrosomonas* and *Nitrosopira*. The tremendous biodiversity among microbes discovered using techniques that do not require culturing (Horner-Devine, Carney & Bohannan 2004; Kassen & Rainey 2004) emphasizes that a very small subset are being

used as microbiosensors. Yet, researchers understandably hope that the bioreporters in use, and derived from commonly engineered species, will provide a microbial 'point of view' of the environment that is in at least some ways generalizable. Basic familiarity with the biology of the microbiosensor strain is essential, and, fortunately, common microbiosensor strains such as various *Pseudomonas* sp., *Bacillus* sp. *S. meliloti*, *E. coli*, and *Erwinia herbicola* have been extensively studied and some of their individual tricks and talents recognized. For example, the ability of *S. meliloti* to take up and use the large and small α -galactosides commonly released from legume seeds argued for its use as a rhizosphere galactoside sensor in soil microcosms (Bringham et al. 2001).

THE GENETIC BACKGROUND OF MICROBIOSENSORS CAN INFLUENCE THEIR ABILITY TO RESPOND TO ENVIRONMENTAL SIGNALS OF INTEREST

Matching of biosensor strains to the question at hand is essential, and a component of that exercise is considering whether the selected promoter is sensitive to the stimulus of interest and whether it will function in the microbiosensor background where it is inserted. Though the diversity of microbiosensor strains is minuscule compared with the diversity of soil micro-organisms, microbiosensor biological diversity is still substantial enough that a promoter taken from one bacterium may not function well in another. We have observed, for example, that the *E. coli proU* promoter moved by Axtell & Beattie (2002) into the related, leaf-dwelling, bacterium *Pantoea agglomerans* functions nicely there and provides inducible activity in the face of changes in water potential in the environment. Yet the same promoter moved into the more distantly related soil bacterium *Pseudomonas putida* has much less activity (Herron 2007).

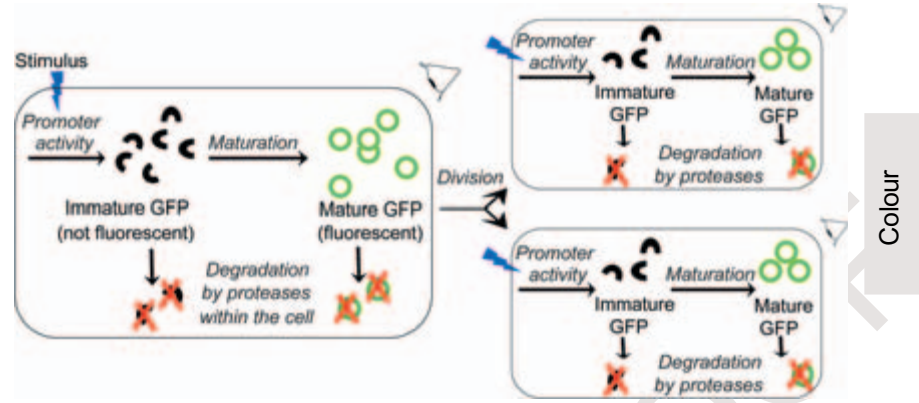
REPORTER GENE EXPRESSION IS A FUNCTION OF MORE THAN JUST STIMULUS INTENSITY AND DYNAMICS

In a microbiosensor the specificity for a particular stimulus and the 'shape' and timing of its response depend on the promoter, the reporter gene and the host biosensor cell. Clearly, a new microbiosensor's response characteristics must be carefully examined under controlled conditions prior to deployment in a complex environment. Those controlled conditions should reflect what is known about the substrates and conditions likely to be experienced by the microbiosensors, as well as their dynamics. It may happen that the reporter gene is expressed in the presence of compounds closely (or not so closely) related to the target stimuli. The *mela* promoter used in Bringham et al. 2001, for instance, which drives expression of an α -galactosidase utilization operon in *S. meliloti*, is also induced to some degree by certain β -galactosides and by galactose (Gage & Long 1998). As a counter-example, the *ara* promoter of *E. coli* has been reported to be induced in a highly specific fashion by arabinose (Daunert et al. 2000).

Sometimes, reporter output may be absent even if the inducing conditions are present. One of these cases may be, as mentioned above, the lack of permissive conditions in the microbe's environment. But this is not the only case. The host micro-organism may not recognize the promoter driving the reporter, or response to the stimulus may vary from one host to the other one, or from one environmental condition to another. Herron (2007) found, for example, that in liquid culture, the expression of GFP under the control of the *proU* promoter in *Pantoea agglomerans* was dramatically changed depending on the carbon substrate (succinate or glucose) provided for growth. Though GFP fluorescence scaled with osmotic potential when either carbon source was present, the scaling was much stronger (the gain in sensor output was much higher) when it was grown in glucose. The same promoter-reporter construct in *Pseudomonas putida* behaved very differently. GFP fluorescence was overall much lower, and scaling of fluorescence was not influenced by the carbon source (Herron 2007). The problem of inherent variability in expression could be further understood if preliminary characterization of biosensors included studies of variability at the single cell level done in parallel with standard, culture-level, expression studies.

Another interesting example of variability in reporting is stochastic gene expression, which may produce heterogeneity of output within a population of microbiosensors (Kaern et al. 2005). Stochasticity results from intrinsic cell-to-cell variability in regulatory factors or in the levels or activity of information-processing proteins. Thus, individual cells in the population, under the same environmental conditions and the same stimulus intensity, can exhibit different output responses. This characteristic may pose a challenge if the response is being recorded at the scale of individual cells, as opposed to being recorded as the aggregate response of many cells measured simultaneously.

Another consideration is whether the promoter drives reporter expression in an 'all or none' fashion, or conversely, in a smoothly graded fashion. Promoters in their native settings are often subjected to the control of regulatory systems that involve feedback. Positive feedback can greatly amplify small differences, creating essentially an on/off switch (Csete & Doyle 2002; Dubnau & Losick 2006). Morgan-Kiss, Wadler & Cronan (2002) suggest that most bacterial expression systems exhibit such 'all-or-nothing' induction. What does this mean in practice, when tracking individual biosensors in the rhizosphere? It means that if the population of biosensors displays such dynamics it will be divided in two groups: one will exhibit the maximum level of expression, whereas the other will not express the reporter signal at all. As the concentration of stimulus increases, a higher proportion of the population turns its switch to the 'on' position, until a threshold is reached at which point all cells emit at their maximum output. Thus, in cases of all-or-nothing responses the strength of the stimulus will be reflected in the ratio of induced to uninduced cells, rather than in the intensity of the response in individual cells. Classical examples of on/off switches are the *lac* and the *ara* operons in *E. coli* (Novick &



Colour

Fig. 1. Diagram of multiple, simultaneous cellular processes influencing the GFP content per cell, and thus fluorescence per cell, for an hypothetical microbiosensor.

Weiner 1957; Khlebnikov *et al.* 2000). In both operons the promoter is induced by a specific molecule (lactose or arabinose), driving the expression of proteins that transport the inducer into the cell. Response to the stimulus (expression of the transport genes) results in a greater capacity for transport of that same stimulus, sending the response into an upward spiral. The outcome is an all-or-nothing response.

Often catabolic genes encoding proteins that degrade the inducer are also under control of the promoter of interest. The presence of these genes may dampen the regulation by lowering the intracellular concentration of the inducer, leading to a graded response (Ozbudak *et al.* 2004). In contrast to these complicated regulatory networks, there are a few relatively simple systems that offer graded responses, such as those that regulate tetracycline resistance (*tetA*) and the porin proteins (*ompF* and *ompC*) in *E. coli* (Batchelor, Silhavy & Goulian 2004).

The inherent complexity of the regulatory circuits that control promoter expression may initially seem to complicate design of the experiments greatly. However, once such idiosyncrasies are identified, characteristics of reporter expression can then be factored into the plan for inoculating bacteria into the environment and interpreting output. Moreover, the presence of many players regulating promoter activity allows for great versatility and flexibility in the engineering of biosensing systems by means of 'choosing and picking' which elements of the regulatory circuit are included (Haseltine & Arnold 2008). For instance, an on/off 'promoter switch' can be converted into a graded-response system by introducing it into a host that lacks some of the regulatory components or by placing the transport genes under the control of an independent promoter (Khlebnikov *et al.* 2000). Similarly, a graded response system may be turned into an on/off one by induction with a 'gratuitous' inducer, one that is not metabolized by the cell (Smits, Kuipers & Veening 2006). The system may even be engineered to produce a step response (Daunert *et al.* 2000; Haseltine & Arnold 2008). The threshold for induction may be manipulated by inclusion or removal of catabolic genes or by incorporation of an activator that responds to a different external stimulus (Daunert *et al.* 2000; Morgan-Kiss *et al.* 2002; Ozbudak *et al.* 2004).

These variations in promoter expression patterns can be manipulated to answer specific questions. The *lac* promoter

can behave as a constitutive promoter in the absence of its repressor, and as such it is used for constitutively labelling cells (Wright & Beattie 2004). This characteristic has also been exploited to report on horizontal gene transfer in the environment, when the donor cells possess a tight repressor and the recipient cells do not (Normander *et al.* 1998). Undetectable signal from low levels of a stimulus, or from low-level gene expression, may be amplified by making the response all-or-nothing and inheritable, such that a single induced cell will give rise to a myriad of descendants that are fully induced (Casavant *et al.* 2002).

Clearly it should not be assumed that expression of the reporter gene is a perfect reflection of promoter activity. Leveau & Lindow (2001) illustrate this with a simple model incorporating steps from promoter activation to folding of GFP into its mature fluorescent form in a hypothetical microbiosensor cell. Figure 1 illustrates the point. Though a stimulus may directly affect promoter activity, other physiological and cellular characteristics also influence the final concentration of GFP per cell. Immature GFP is made after the promoter is induced and mRNA is transcribed, and it has several potential fates (Fig. 1). Some is degraded; some matures to visible GFP. If and when the cell divides, the pools of GFP are divided into the daughter cells, instantaneously reducing the cellular content by half. Fluorescence of mature GFP per cell is thus a complex function of all these simultaneous processes, as well as a function of variations in promoter activity in response to signal.

Figure 1 also hints at a final point. The researcher's eye more easily sees green fluorescent protein, potentially biasing against detection of biosensor cells that are not induced (e.g. Leveau & Lindow 2002). Not only is it very important to make sure that if there is a signal it will be detected and interpreted correctly, it also is very important to use methods discussed earlier to insure that both induced and uninduced sensors are tracked.

THE BLESSING AND CURSE OF CELL DIVISION

As suggested in Fig. 1, the physiological complexity potentially muddying the correspondence between GFP content per cell and stimulus intensity can also be viewed as an opportunity. Long-lived GFP molecules are useful in soil,

1 where it can be extremely difficult to see fluorescence from
 2 green fluorescent protein. By using the long-lived variant of
 3 GFP, there is better opportunity to detect a GFP signal from
 4 cells because it is brighter. A number of GFP variants with
 5 different turnover times are available for use as reporter
 6 molecules (Andersen *et al.* 1998), and fast-turnover GFP
 7 would appear to provide a more dynamic signal that might
 8 facilitate tracking rapid changes in soil, or other complex
 9 environments. However, overall fluorescence levels of fast-
 10 turnover GFP per cell are lower. In complex environments
 11 such as soil, such diminished fluorescence could pose a
 12 significant difficulty for signal detection. On the other hand,
 13 intuitively it seems that by using a long-lived GFP molecule
 14 that builds up in cells, and does not degrade readily, the ability
 15 of the researcher to detect dynamic changes in promoter
 16 activity would be swamped out by large, permanent, pools of
 17 GFP. This problem is mentioned in Hansen & Sørensen's
 18 (2001) review. However, it is possible to use long-lived report
 19 molecules to detect relatively rapid dynamics in stimulus
 20 intensity *if* the population of microbiosensor bacteria is
 21 growing rapidly. Referring to Fig. 1, note that if cells are
 22 dividing quickly, variations in promoter activity can be
 23 rapidly translated into detectable changes in mature GFP per
 24 cell because rapid growth and cell division continually divide
 25 the number of GFP molecules per cell. Large, permanent
 26 pools have less opportunity to build up during rapid growth.
 27 For tracking dynamics and spatial patterns of rhizosphere
 28 water potential or sugar availability, this coupling could be
 29 essential. Fortunately, the rhizosphere is known as a hotbed
 30 of microbial activity, where, unlike in bulk soil, carbon is
 31 readily available to support microbial growth. Studies of
 32 microbial growth rates in carbon-amended soil suggest that
 33 growth rates as high as one doubling per 2 h may be possible
 34 in carbon-replete zones such as might be found in the rhizo-
 35 sphere (Behera & Wagner 1974). Microbiosensors reporting
 36 bacterial growth rates (e.g. Ramos *et al.* 2000) at different
 37 zones along roots could also help guide design and interpreta-
 38 tion of experiments, where the advantages of brightness of
 39 the overall GFP signal must be balanced with enhanced
 40 ability to detect a fluctuating GFP signal on a low fluores-
 41 cence background.

42 43 44 CAN MICROBIOSENSORS REPORT QUANTITATIVELY IN 45 COMPLEX ENVIRONMENTS?

46 Hansen & Sørensen's (2001) review emphasizes micro-
 47 biosensor function in the context of sensing specific and
 48 non-specific environmental stresses, for example sensing toxic
 49 compounds in extracts, products (e.g. media, milk), and the
 50 environment. In well-defined, homogeneous, contexts more
 51 control over the environment of the sensors can be exerted
 52 and microbiosensors' output can sometimes be quantitatively
 53 interpreted. But in complex, heterogeneous, environments
 54 where energy sources and nutrient availabilities and conditions
 55 are not known at microbial spatial scales, exact quantitation
 56 of stimuli *in situ* seems doubtful. The capabilities of several
 57 microbiosensors may be required to provide an envelope in

which to interpret spatial and temporal variation in micro-
 biosensor responses *in situ*. The reporters mentioned by
 Hansen and Sørensen in which *lux* genes, for example, are
 constitutively expressed, and light production is used as an
 indicator of stress or lack of metabolic activity, provide an
 intriguing possibility for use in the microbial ecologist's
 toolkit. Such an indicator of metabolic activity could be
 harnessed to show subtle variations in availability of e.g.
 energy in the environment, and perhaps provide useful
 information on the capacity, in general, of those microbes to
 respond to other stimuli measured by other reporters linked
 to inducible genes.

MICROBIOSENSORS ARE LUNCH

Finally, it can't be ignored that microbiosensors are living
 bacteria and thus enter the food chain in non-sterile soils
 (Clarholm 2002; Bonkowski 2004; Cardon & Gage 2006;
 Moore, McCann & de Ruiter 2007). Microbiosensor popu-
 lation size and signal intensity decrease over time with
 predation, but this sobering fact can also be seen in a positive
 light. It is extremely difficult to examine predation in the soil
 environment, but protozoal grazing on fluorescently-tagged
 bacteria is a promising way to track predation at extremely
 small scales, because the fluorescent proteins can continue
 to provide signal even after reporter bacteria are engulfed
 (Bringhurst *et al.* 2001).

Conclusion

In sum, microbiosensors provide an exciting gene-based way
 to probe microbial perception of local soil environments, and
 microbial responses to those environments, continuously and
 at high spatial resolution. As with all methods, there are
 tradeoffs associated with using microbiosensors, tradeoffs
 stemming from their basic biology and their diversity. Still,
 the promise that microbiosensors hold for orienting us to the
 microbial perception of the soil environment, and for reveal-
 ing mechanistic themes of organismal interactions in and
 with soil, becomes more exciting as additional links among
 microbial activity, community structure, and belowground
 ecosystem functions are forged.

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References

- Andersen, J.B., Sternberg, C., Poulsen, L.K., Bjorn, S.P., Givskov, M. &
 Molin S (1998) New unstable variants of green fluorescent protein for
 studies of transient gene expression in bacteria. *Applied and Environmental
 Microbiology*, **64**, 2240–2246.

- 1 Axtell, C.A. & Beattie G.A. (2002) Construction and characterization of a
 2 *proU-gfp* transcriptional fusion that measures water availability in a microbial
 3 habitat. *Applied and Environmental Microbiology*, **68**, 4604–4612.
- 4 Batchelor, E., Silhavy, T.J. & Goulian, M. (2004) Continuous control in
 5 bacterial regulatory circuits. *Journal of Bacteriology*, **186**, 7618–7625.
- 6 Behera, B. & Wagner, G.H. (1974) Microbial growth rate in glucose-amended
 7 soil. *Soil Science Society of America Journal*, **38**, 591–594.
- 8 Boldt, T.S., Sorensen, S.J., Karlson, U., Molin, S. & Ramos, C. (2004)
 9 Combined use of different Gfp reporters for monitoring single-cell activities
 10 of a genetically modified PCB degrader in the rhizosphere of alfalfa. *FEMS
 11 Microbiology Ecology*, **48**, 139–148.
- 12 Bonkowski, M. (2004) Protozoa and plant growth: the microbial loop in soil
 13 revisited. *New Phytologist*, **162**, 617–631.
- 14 Bringham, R.M., Cardon, Z.G. & Gage, D.J. (2001) Galactosides in the
 15 rhizosphere: utilization by *Sinorhizobium meliloti* and development of a
 16 biosensor. *Proceedings of the National Academy of Sciences USA*, **98**, 4540–
 17 4545.
- 18 Cardon, Z.G. & Gage, D.J. (2006) Resource exchange in the rhizosphere:
 19 molecular tools and the microbial perspective. *Annual Review of Ecology,
 20 Evolution, and Systematics*, **37**, 459–488.
- 21 Cardon, Z.G., Czaja, A.D., Funk, J.L. & Vitt, P.L. (2002) Periodic carbon
 22 flushing to roots of *Quercus rubra* saplings affects soil respiration and rhizo-
 23 sphere microbial biomass. *Oecologia*, **133**, 215–223.
- 24 Casavant, N.C., Beattie, G.A., Phillips, G.J. & Halverson, L.J. (2002) Site-
 25 specific recombination-based genetic system for reporting transient or
 26 low-level gene expression. *Applied and Environmental Microbiology*, **68**,
 27 3588–3596.
- 28 Clarholm, M. (2002) Bacteria and protozoa as integral components of the for-
 29 est ecosystem their role in creating a naturally varied soil fertility. *Antonie
 30 Van Leeuwenhoek International Journal of General and Molecular Microbi-
 31 ology*, **81**, 309–318.
- 32 Crawford, J.W., Harris, J.A., Ritz, K. & Young, I.M. (2005) Towards an
 33 evolutionary ecology of life in soil. *Trends in Ecology and Evolution*, **20**, 81–87.
- 34 Csete, M.E. & Doyle, J.C. (2002) Reverse engineering of biological complexity.
 35 *Science*, **295**, 1664–1669.
- 36 Daunert, S., Barrett, G., Feliciano, J.S., Shetty, R.S., Shrestha, S. &
 37 Smith-Spencer, W. (2000) Genetically engineered whole-cell sensing systems:
 38 coupling biological recognition with reporter genes. *Chemical Reviews*, **100**,
 39 2705–2738.
- 40 DeAngelis, K.M., Firestone, M.K. & Lindow, S.E. (2007) Sensitive whole-cell
 41 biosensor suitable for detecting a variety of N-acyl homoserine lactones in
 42 intact rhizosphere microbial communities. *Applied and Environmental
 43 Microbiology*, **73**, 3724–3727.
- 44 DeAngelis, K.M., Ji, P.S., Firestone, M.K. & Lindow, S.E. (2005) Two novel
 45 bacterial biosensors for detection of nitrate availability in the rhizosphere.
 46 *Applied and Environmental Microbiology*, **71**, 8537–8547.
- 47 Dubnau, D. & Losick, R. (2006) Bistability in bacteria. *Molecular Microbiology*,
 48 **61**, 564–572.
- 49 Gage, D.J. & Long, S.R. (1998) α -galactoside uptake in *Rhizobium meliloti*: iso-
 50 lation and characterization of *agpA*, a gene encoding a periplasmic binding
 51 protein required for melibiose and raffinose utilization. *Journal of Bacteri-
 52 ology*, **180**, 5739–5748.
- 53 Griffiths, B.S., Christensen, S. & Bonkowski, M. (2007) Microfaunal interactions
 54 in the rhizosphere – how nematodes and protozoa link above- and below-
 55 ground processes. *The Rhizosphere: An Ecological Perspective* (eds Z.G.
 56 Cardon & J.L. Whitbeck), pp. 57–72. Elsevier, San Diego.
- 57 Hansen, L.H. & Sorensen, S.J. (2001) The use of whole-cell biosensors to
 detect and quantify compounds or conditions affecting biological systems.
Microbial Ecology, **42**, 483–494.
- Haseltine, E.L. & Arnold, F.H. (2008) Implications of rewiring bacterial
 quorum sensing. *Applied and Environmental Microbiology*, **74**, 437–445.
- Herron, P.M. (2007) *Vapors, Fluorescence, and Bioluminescence: New Approaches
 and Insights into Plant and Microbial Processes in the Rhizosphere*. PhD
 thesis, University of Connecticut, Storrs.
- Hillel, D. (1998) *Environmental Soil Physics*. Academic Press, San Diego.
- Horner-Devine, M.C., Carney, K.M. & Bohannan, B.J.M. (2004) An ecological
 perspective on bacterial biodiversity. *Proceedings of the Royal Society of
 London Series B*, **271**, 113–122.
- Jaeger, C.H., Lindow, S.E., Miller, S., Clark, E. & Firestone, M.K. (1999)
 Mapping of sugar and amino acid availability in soil around roots with
 bacterial sensors of sucrose and tryptophan. *Applied and Environmental
 Microbiology*, **65**, 2685–2690.
- Jones, D.L., Hodge, A. & Kuzyakov, Y. (2004) Plant and mycorrhizal regula-
 tion of rhizodeposition. *New Phytologist*, **163**, 459–480.
- Kaern, M., Elston, T.C., Blake, W.J. & Collins, J.J. (2005) Stochasticity in gene
 expression: from theories to phenotypes. *Nature Reviews Genetics*, **6**, 451–
 464.
- Kassen, R. & Rainey, P.B. (2004) The ecology and genetics of microbial
 diversity. *Annual Review of Microbiology*, **58**, 207–231.
- Khlebnikov, A., Risa, O., Skaug, T., Carrier, T.A. & Keasling, J.D. (2000)
 Regulatable arabinose-inducible gene expression system with consistent
 control in all cells of a culture. *Journal of Bacteriology*, **182**, 7029–7034.
- Leveau, J.H.J. & Lindow, S.E. (2001) Predictive and interpretive simulation
 of green fluorescent protein expression in reporter bacteria. *Journal of
 Bacteriology*, **183**, 6752–6762.
- Leveau, J.H.J. & Lindow, S.E. (2002) Bioreporters in microbial ecology.
Current Opinion in Microbiology, **5**, 259–265.
- Lewenza, S., Falsafi, R.K., Winsor, G., Gooderham, W.J., McPhee, J.B., Brinkman,
 F.S.L. & Hancock, R.E.W. (2005) Construction of a mini-Tn5-*luxCDABE*
 mutant library in *Pseudomonas aeruginosa* PAO1: a tool for identifying
 differentially regulated genes. *Genome Research*, **15**, 583–589.
- Moore, J.C., McCann, K. & de Ruiter, P.C. (2007) Soil rhizosphere food webs,
 their stability, and implications for soil processes and ecosystems. *The
 Rhizosphere: An Ecological Perspective* (eds Z.G. Cardon & J.L. Whitbeck),
 pp. 101–126. Elsevier, San Diego.
- Morgan-Kiss, R.M., Wadler, C. & Cronan, J.E. Jr. (2002) Long-term and
 homogeneous regulation of the *Escherichia coli* *araBAD* promoter by use of
 a lactose transporter of relaxed specificity. *Proceedings of the National
 Academy of Sciences USA*, **99**, 7373–7377.
- Normander, B., Christensen, B.B., Molin, S. & Kroer, N. (1998) Effect of
 bacterial distribution and activity on conjugal gene transfer on the
 phylloplane of the bush bean (*Phaseolus vulgaris*). *Applied and Environ-
 mental Microbiology*, **64**, 1902–1909.
- Novick, A. & Weiner, M. (1957) Enzyme induction as an all-or-none phenomenon.
Proceedings of the National Academy of Sciences USA, **43**, 553–566.
- Ozбудak, E.M., Thattai, M., Lim, H.N., Shraiman, B.I. & Van Oudenaarden,
 A. (2004) Multistability in the lactose utilization network of *Escherichia coli*.
Nature, **427**, 737–740.
- Ramos, C., Molbak, L. & Molin, S. (2000) Bacterial activity in the rhizosphere
 analyzed at the single-cell level by monitoring ribosome contents and
 synthesis rates. *Applied and Environmental Microbiology*, **66**, 801–809.
- Ratray, E.A.S., Prosser, J.I., Glover, L.A. & Killham, K. (1995) Characterization
 of rhizosphere colonization by luminescent *Enterobacter cloacae* at the
 population and single-cell levels. *Applied and Environmental Microbiology*,
61, 2950–2957.
- Read, D.J. & Perez-Moreno, J. 2003. Mycorrhizas and nutrient cycling in
 ecosystems – a journey towards relevance? *New Phytologist*, **157**, 475–
 492.
- Schimel, J.P. & Bennett, J. (2004) Nitrogen mineralization: challenges of a
 changing paradigm. *Ecology*, **85**, 591–602.
- Smits, W.K., Kuipers, O.P. & Veening, J.W. (2006) Phenotypic variation in
 bacteria: the role of feedback regulation. *Nature Reviews in Microbiology*, **4**,
 259–271.
- Wright, C.A. & Beattie, G.A. (2004) Bacterial species specificity in *proU*
 osmoinducibility and *nptII* and *lacZ* expression. *Journal of Molecular and
 Microbiological Biotechnology*, **8**, 201–208.
- Young, I.M. & Ritz, K. (1998) Can there be a contemporary ecological dimension
 to soil biology without a habitat? Discussion. *Soil Biology and Biochemistry*,
30, 1229–1232.

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