# Bioavailability of soil organic carbon and Fe as influenced by forestry practices in a subtropical coastal catchment

**Chaofeng Lin**, Eloise Larsen, Peter Grace and James Smith

Faculty of Science and Technology & Institute for Sustainable Resources, Queensland University of Technology, Brisbane, QLD, Australia, Email linc3@qut.edu.au

## Abstract
Potential impacts of plantation forestry practices on soil organic carbon and Fe available to microorganisms were investigated in a subtropical coastal catchment. The impacts of harvesting or replanting were largely limited to the soil top layer (0–10 cm depth). The thirty-year-old *Pinus* plantation showed low soil moisture content (Wc) and relatively high levels of soil total organic carbon (TOC). Harvesting and replanting increased soil Wc but reduced TOC levels. Mean dissolved organic carbon (DOC) and microbial biomass carbon (MBC) increased in harvested or replanted soils, but such changes were not statistically significant ($P > 0.05$). Total dithionite-citrate and aqua regia-extractable Fe did not respond to forestry practices, but acid ammonium oxalate and pyrophosphate-extractable, bioavailable Fe decreased markedly after harvesting or replanting. Numbers of heterotrophic bacteria were significantly correlated with DOC levels ($P < 0.05$), whereas Fe-reducing bacteria and S-bacteria detected using laboratory cultivation techniques did not show strong correlation with either soil DOC or Fe content.

## Keywords
Subtropical catchment; forestry management; soil carbon; Fe biogeochemistry; soil bacteria.

## Introduction
Previous research suggests that forestry managements can affect rates of elemental cycling, nutrient availability, as well as microbial community structure in soils by changing soil moisture content (Wc) and clay content, as well as pH and other physicochemical factors (Buzek, Paces *et al.* 2009; Frey, Kremer *et al.* 2009; Kara and Bolat 2008). Labile soil organic carbon indicators such as dissolved organic carbon (DOC) and microbial biomass carbon (MBC) are often used to assess soil quality, particularly soil organic carbon (SOC) changes, as they provide a relatively short-term response to changes in soil management, as compared with soil total organic carbon (TOC). This study was conducted in a subtropical coastal catchment undergoing active plantation forestry practices, e.g. harvesting and replanting. We aimed to determine the microbiological bioavailability of SOC in response to forestry practices by examining DOC and MBC in different soils. A regional concern is that potentially enriched SOC may promote Fe dissolution and subsequent mobilisation, either chemically or microbially, from catchment soils into the estuary ecosystem. Enhanced elemental cycling in such habitats can be driven substantially by biogeochemical processes in which microbial activities are major catalysts (Stemmler and Berthelin 2003). To assess the potential for Fe release from soils into the surrounding waterways, Fe (active, bioavailable and dissolved forms) was extracted and related bacterial populations were enumerated using laboratory cultivation techniques.

## Methods
*Study area and soil sampling*
The Poona Catchment (150 $km^2$) is on a relatively flat coastal plain, located ca. 300 km north of Brisbane on the Fraser Coast of SE QLD (Australia), discharging into the Great Sandy Strait, an environmentally-sensitive estuarine habitat of national significance, as well as a UNESCO-listed world heritage area. The catchment has a high summer and low winter rainfall due to the subtropical climate. The annual average rainfall is 1270 mm. The mean monthly maximum temperatures range from 30.2ºC in Dec. to 21.5ºC in July. Presently, the dominant land-use is *Pinus* plantation forestry (58 $km^2$). Forest soils were sampled from three sites (ca. 30-year-old forested, OF; newly-harvested, NH; and newly-planted, NP) in the upper-catchment in Dec., 2008. Intact duplicate 30 cm push-cores were collected at each site using PVC tubing, and were sealed for transportation to the laboratory within two days. Fresh cores were separated into three segments (0–10 cm, top layer; 10–20 cm, mid layer and 20–30 cm, bottom layer) and homogenized before analysis. Replicate subsamples were processed for microbial analysis within two weeks. The remaining subsamples were stored at 4ºC for soil physicochemical analysis.

*Soil physicochemical and microbial analysis*

Soil physicochemical properties were determined as follows: soil Particle Volume Distribution (PVD) by laser particle sizing; soil Wc, gravimetrically on drying (105ºC, 24h); pH, redox potential (Eh) and electron conductivity (Ec) in 1:5 soil:water suspension using a TPS multiple field analyser (90-FMLV); soil dissolved nitrate, phosphate, sulfate and Fe by 0.01M $CaCl_2$-extraction (Houba, Temminghoff *et al.* 2000); TOC by Loss-On-Ignition (500ºC, 4h); DOC by cold water-extraction (Robertson, Coleman *et al.* 1999); MBC by chloroform-fumigation-extraction (Vance, Brookes *et al.* 1987); and total reactive Fe by dithionite citrate-extraction ($Fe_{DC}$), aqua regia-extraction ($Fe_{aqua}$), acid ammonium oxalate-extraction ($Fe_{AAO}$) and pyrophosphate-extraction ($Fe_{pyro}$) (Courchesne and Turmel 2007). For $Fe_{CaCl2}$ and $Fe_{AAO}$ extraction, anaerobic techniques were used (Phillips and Lovely 1986). Extracts were analysed within two weeks of extraction. For cultivation and enumeration of bacteria potentially involved in Fe cycling, selective enrichment media were used (Table 1). Bacterial numbers per gram soil were determined by a 1:10 dilution-to-extinction method. R2A medium was used to enumerate heterotrophic bacteria (colony-forming units, CFU) by the plate count method (HPC). Cultures were collected after 4 weeks incubation.

**Table 1. Selective enrichment media used for cultivation and enumeration of Fe cycle-related bacteria**

| Medium | pH | Target bacteria | Reference |
|---|---|---|---|
| 9K | 2.5 | Acidophilic Fe(II)-oxidising (FeOB) | Eaton, Clesceri *et al.* 2005 |
| Liquid gradient medium | 4.8 | Neutrophilic, microaerophilic FeOB | Hanert 2006 |
| Semi-solid gradient medium | 6.3 | Neutrophilic, microaerophilic FeOB | Emerson and Floyd 2005 |
| Fe(III)-$NO_3$ medium | 7.0 | Anaerobic, nitrate-dependent FeOB | Widdel and Bak 1992 |
| Fe(III)-EDTA medium | 7.0 | Neutrophilic Fe(III)-reducing (FeRB) | Gould, Stitchbury *et al.* 2003 |
| Sulfur medium | 4.8 | Sulfur-oxidising (SOB) | Eaton, Clesceri *et al.* 2005 |
| Thiosulfate medium | 7.8 | Thiosulfate-oxidising (TOB) | Eaton, Clesceri *et al.* 2005 |
| MP liquid medium | 7.0 | Sulfide-oxidising (MPB) | Eaton, Clesceri *et al.* 2005 |
| API medium | 7.5 | Sulfate-reducing (SRB) | Eaton, Clesceri *et al.* 2005 |

*Data analysis*

Data were subjected to GLM ANOVA, correlation and regression analysis using SPSS 16.

## Results

*Soil physicochemical properties*

Based on PVD analysis (Table 2), all soils were classified as loamy sand. Soil Wc was low in site OF, i.e. 3.97–5.10% in soil profile. But at site NH and NP, soil Wc increased to 6.56–7.56% and 9.18–13.13%, respectively. All samples were weakly-acidic. The top layer soil pH at site OF was ca. 6.2 which decreased to ca. 5.1 and 5.3 at site NH and NP. In contrast, the pH in mid and bottom layer soils did not vary between treatments as much as the top layer soil. The Eh remained at 288–336 mV for all soils, indicating a well-oxidised environment regardless of forestry practice. Soil Ec did not respond to forestry practice. Dissolved nutrient analysis revealed low soil fertility at site OF. By comparison, dissolved nitrate in NH soils, increased markedly, but appeared to decrease in NP soils, displaying similar levels to those in mature forested soils. Dissolved phosphate was seldom detected, while sulfate appeared not to be limiting in Poona catchment.

**Table2. Soil physicochemical properties**

| Site | Depth | PVD (%) | | | Wc | pH | Eh | Ec | Soil dissolved nutrients (µg/g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | (cm) | Clay | Silt | Sand | (%) | | (mV) | (µS/cm$^2$) | $NO_3$-N | $PO_4$-P | $SO_4$-S |
| OF | 0–10 | 6.8 | 14.4 | 78.8 | 4.0 | 6.2 | 288 | 17.0 | 2 | 0 | 26 |
| | 10–20 | 8.2 | 15.6 | 76.3 | 4.5 | 6.1 | 301 | 8.1 | 2 | 0 | 18 |
| | 20–30 | 8.8 | 18.3 | 72.9 | 5.1 | 6.0 | 312 | 7.7 | 2 | 0 | 27 |
| NH | 0–10 | 4.7 | 14.0 | 81.3 | 6.6 | 5.1 | 336 | 19.0 | 7 | 0.3 | 38 |
| | 10–20 | 5.8 | 13.6 | 80.6 | 6.9 | 5.5 | 325 | 8.6 | 5 | 0.3 | 28 |
| | 20–30 | 4.8 | 12.8 | 82.4 | 7.6 | 5.8 | 319 | 7.9 | 5 | 0 | 22 |
| NP | 0–10 | 2.9 | 14.5 | 82.6 | 13.1 | 5.3 | 309 | 7.2 | 3 | 1.6 | 17 |
| | 10–20 | 5.1 | 12.8 | 82.0 | 8.6 | 5.8 | 296 | 4.4 | 3 | 0.05 | 20 |
| | 20–30 | 7.6 | 13.4 | 79.0 | 9.2 | 6.1 | 327 | 4.9 | 3 | 0 | 16 |

*SOC and Fe analysis*

Soil TOC (Table 3) at site OF was ca. 2.7–2.9% with a uniform distribution in soil profile. However, TOC from NH (1.0–1.5%) and NP (0.7–1.9%) soil was significantly lower ($P < 0.05$). Soil available organic carbon, i.e. DOC and MBC peaked in top layer soils, 50–70 µg/g and 167–246 µg/g, respectively. DOC was

higher in both NH and NP soils as compared with OF soils. In contrast, MBC was highest at site NP, particularly in top layer soils. A positive correlation between DOC and MBC was shown ($F = 0.641$, $P < 0.01$, $n = 18$).

**Table 3. SOC and extractable Fe (µg/g dry soil)**

| Site | Depth (cm) | TOC (%) | DOC | MBC | $Fe_{DC}$ | $Fe_{aqua}$ | $Fe_{AAO}$ | $Fe_{pyra}$ | $Fe_{CaCl2}$ |
|---|---|---|---|---|---|---|---|---|---|
| OF | 0–10 | 2.9 | 50 | 193 | 5031 | 3316 | 1735 | 1791 | 0.403 |
| | 10–20 | 2.7 | 18 | 174 | 6967 | 3280 | 2273 | 2069 | 0.253 |
| | 20–30 | 2.8 | 4 | 100 | 9450 | 5999 | 3698 | 3190 | 0.469 |
| NH | 0–10 | 1.5 | 70 | 167 | 4696 | 2445 | 665 | 798 | 0.469 |
| | 10–20 | 1.0 | 21 | 55 | 10478 | 3526 | 684 | 586 | 0.372 |
| | 20–30 | 1.0 | 14 | 35 | 7306 | 2452 | 618 | 706 | 0.423 |
| NP | 0–10 | 1.9 | 64 | 246 | 4534 | 1169 | 530 | 469 | 0.911 |
| | 10–20 | 0.7 | 11 | 102 | 5885 | 1709 | 704 | 619 | 0.534 |
| | 20–30 | 1.0 | 6 | 24 | 6634 | 4434 | 1069 | 1116 | 0.271 |

Soil total active (fine crystal, poorly crystalline and organically-complexed) Fe extracted with dithionite citrate ($Fe_{DC}$) yielded the highest concentration, ca. 1.5–4 times that of $Fe_{aqua}$, yet results from the two methods showed correlation at significant level ($n=18$, $F = 0.77$, $P < 0.01$). Both $Fe_{DC}$ and $Fe_{aqua}$ increased with depth instead of responding to harvesting or planting, whereas microbially bioavailable Fe (poorly crystalline and organically complexed, $Fe_{AAO}$ and $Fe_{pyra}$) was more sensitive to forest practices. For example, at site OF, $Fe_{AAO}$ ranged from 1735 to 3698 µg/g soil in the profile, but decreased markedly in NH soils (618–665 µg/g) and NP soils (530–1069 µg/g). In contrast, total dissolved Fe ($Fe_{CaCl2}$) was distributed in a different way. $Fe_{CaCl2}$ was increased particularly in site NP, but such a difference was not statistically significant.

*Fe biogeochemistry-related bacterial populations*

Bacteria are important catalysts of soil organic matter decay and elemental cycling. In Poona catchment forest soils, heterotrophic bacteria were found at ca. $10^6$ CFU/g dry soil (Table 4). Bacterial cell numbers were significantly correlated with DOC ($F = 0.63$, $P < 0.05$, $n = 18$), indicating that soil available organic carbon is a major controlling factor in the distribution of heterotrophs. Previous research also showed that soil biota tend to increase in the first few years after clear cutting (Paul and Clark 1996). Factors such as availability of dead roots for decomposition, greater susceptibility of residual litter to decomposition due to more favourable soil Wc, temperature, and regrowth of herbs or shrubs all contribute to the increased SOC.

**Table 4. Bacteria cultivated from forest soils ($log_{10}$ CFU/g or cell/g dry soil)**

| Site | Depth (cm) | Heterotrophic bacteria | Fe-bacteria | | | | S-bacteria | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $FeOB_3$[a] | $FeOB_7$[b] | $FeOB_{NO3}$[c] | FeRB | MPB | SOB | TOB | SRB |
| OF | 0–10 | 6.1 | ND[d] | ND | ND | 6.5 | 3.5 | 1.0 | 1.5 | ND |
| | 10–20 | 6.0 | ND | ND | ND | 7.0 | 1.5 | 1.0 | 1.0 | ND |
| | 20–30 | 5.8 | ND | ND | ND | 7.5 | 2.0 | 0.5 | 1.0 | ND |
| NH | 0–10 | 6.6 | ND | ND | ND | 7.0 | 2.5 | 3.5 | 1.5 | ND |
| | 10–20 | 6.1 | ND | ND | ND | 7.0 | 1.5 | 2.5 | 1.0 | ND |
| | 20–30 | 6.4 | ND | ND | ND | 6.5 | 2.0 | 1.5 | 1.0 | ND |
| NP | 0–10 | 6.2 | ND | ND | ND | 9.0 | 2.0 | 4.5 | 1.5 | ND |
| | 10–20 | 5.8 | ND | ND | ND | 6.0 | 1.0 | 3.0 | 2.5 | ND |
| | 20–30 | 5.6 | ND | ND | ND | 5.5 | 1.0 | 2.5 | 2.0 | ND |

a. acidophilic FeOB; b. neutrophilic, microaerophilic FeOB; c. anaerobic, nitrate-dependent FeOB; d. not detected.

As the soil pH ranged from 5.1–6.2, it was not surprising that no acidophilic FeOB were detected through laboratory cultivation. However, neither neutrophilic FeOB (microaerobic or anaerobic, nitrate-dependent) were detectable in soils using a variety of selective enrichment media. In contrast, neutrophilic FeRB ($> 10^5$ cell/g dry soil) were found in all soils, suggesting that over the duration of the sampling period, microbial Fe(III) reduction coupled with chemical Fe(II) oxidation dominates Fe biogeochemistry. FeRB numbers in top layer soils followed the order NP > NH > OF (Table 4), but did not respond to forestry practices in mid or bottom layer soils. No significant correlation was found between FeRB and extractable Fe of any kind. As the soil pH was weakly acid (ca. 5.5 or less), the Fe(III) concentration may not be limiting to FeRB. A differentiation of Fe species and bacterial quantification by molecular techniques would help to further describe FeRB distribution.

Fe turnover in oxic-anoxic transition zones with circumneutral pH may be highly active. In our case, dissolved Fe(II) in near neutral pH soil solutions may be rapidly, abiotically oxidised, while subsequently-produced amorphous Fe(III) compounds can be utilised immediately by FeRB as a preferred substrate. S-bacteria, particularly SRB, are often involved in Fe cycling in natural habitats (Lovley 2006). Surprisingly, three groups of S-bacteria capable of oxidising sulfide, sulfur or thiosulfate were detected in many soils but no SRB were detected (Table 4). Since sulfate appeared not to be limiting in this area, the absence of SRB may be attributed to the highly oxidised soil conditions. The coexistence of FeRB and diverse SOBs in the absence of FeOB or SRB implies a complex soil-Fe-bacteria ecosystem in which abiotic and biotic processes are interacting. Further DNA-based analysis (in progress) is required to identify the specific organism associated with these cultures.

**Conclusion**

In Poona catchment, harvesting and replanting of *Pinus* plantation forests affects soil pH, Wc and other physicochemical properties. Such impacts are largely limited to the top soil layer. Soil TOC was decreased in NH and NP soils, whereas soil DOC and MBC were increased. In contrast, total active Fe ($Fe_{DC}$ and $Fe_{aqua}$) did not respond to forestry practices, while bioavailable Fe ($Fe_{AAO}$ and $Fe_{pyra}$) decreased markedly in NH and NP soils. The numbers of heterotrophic bacteria were correlated with soil DOC content, but neither culturable Fe-reducing bacteria nor S-bacteria were correlated with SOC or extractable Fe of any kind. Nevertheless, NH or NP soils appeared to harbour higher numbers of these bacteria, indicating a more active soil-Fe-bacteria ecosystem. Our study detected changes in the bioavailability of SOC and Fe due to forestry practices, which further affected microbial populations potentially involved in Fe biogeochemistry.

**Reference**


Buzek F, Paces T, Jackova I (2009) Production of dissolved organic carbon in forest soils along the north-south European transect. *Applied Geochemistry* **24**(9), 1686-1701.

Courchesne F, Turmel M-C (2007) Extractable Al. Fe, Mn, and Si. In 'Soil Sampling and Methods of Analysis.' 2nd edn. (Eds MR Carter and EG Gregorich) pp. 307-350. (CRC Press: Boca Raton)

Eaton AD, Clesceri LS, Rice EW, Greenberg AE (Eds) (2005) 'Standard methods for the examination of water and wastewater (21th edn).' (APHA-AWWA-WEF: Washington, DC)

Emerson D, Floyd MM (2005) Enrichment and isolation of iron-oxidizing bacteria at neutral pH. *Methods in enzymology* **397**, 112-123.

Frey B, Kremer J, Rüdt A, Sciacca S, Matthies D, Lüscher P (2009) Compaction of forest soils with heavy logging machinery affects soil bacterial community structure. *European Journal of Soil Biology* **45**(4), 312-320.

Gould WD, Stitchbury M, Francis M, Lortie L, Blowes DW An MPN method for the enumeration of iron-reducing bacteria. In 'Mining and the Environment Conference', 2003, Sudbury, Canada. (Eds G Spiers, P Beckett and H Conroy)

Hanert H (2006) The genus *Gallionella*. In 'The Prokaryotes. Vol. 7.' (Eds MM Dworkin, K-H Schleifer, E Rosenberg and S Falkow) pp. 990-995. (Springer-Verlag: New York)

Houba VJG, Temminghoff EJM, Gaikhorst GA, Van Vark W (2000) Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Communications in Soil Science and Plant Analysis* **31**(9), 1299-1396.

Kara O, Bolat I (2008) Soil microbial biomass C and N changes in relation to forest conversion in the Northwestern Turkey. *Land Degradation & Development* **19**(4), 421-428.

Lovley D (2006) Dissimilatory Fe(III)- and Mn(IV)-reducing prokaryotes. *The Prokaryotes* **2**, 635-658.

Paul EA, Clark FE (Eds) (1996) 'Soil microbiology and biochemistry (2nd edn).' (Academic Press San Diego)

Phillips EJP, Lovely DR (1986) Determination of Fe(III) and Fe(II) in oxalate extracts of sediment. *Soil Science Society of America Journal* **51**, 938-941.

Robertson GP, Coleman DC, Bledsoe CS, Sollines P (1999) 'Standard soil methods for long-term ecological research.' (Oxford University Press: New York)

Stemmler SJ, Berthelin J (2003) Microbial activity as a major factor in the mobilization of iron in the humid tropics. *European Journal of Soil Science* **54**(4), 725-733.

Vance E, Brookes P, Jenkinson D (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* **19**(6), 703-707.

Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In 'The Prokaryotes. Vol. 4.' (Eds A Ballows, HG Truper, M Dworkin, W Harder and KH Shleifer) pp. 3352-3378. (Springer: Berlin).