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Occurrence of iron and associated bacterial populations in soils of a forested subtropical coastal catchment (*Iron and iron bacteria in catchment soils*)

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Abstract Iron (Fe) biogeochemistry is potentially of environmental significance in plantation-forested, subtropical coastal ecosystems where soil disturbance and seasonal water-logging may lead to elevation of Fe mobilization and associated water quality deterioration. Using wet-chemical extraction and laboratory cultivation, we examined the occurrence of Fe forms and associated bacterial populations in diverse soils of a representative subtropical Australian coastal catchment (Poona Creek). Total reactive Fe was abundant throughout 0–30 cm soil cores, consisting primarily of crystalline forms in well-drained sand soils and water-logged loam soils, whereas in water-logged, low-clay soils, over half of total reactive Fe was present in poorly-crystalline forms due to organic and inorganic complexation, respectively. Forestry practices such as plantation clear-felling and replanting, seasonal water-logging and mineral soil properties significantly impacted soil organic carbon (C), potentially-bioavailable Fe pools and densities of S-, but not Fe-, bacterial populations. Bacterial Fe(III) reduction and abiotic Fe(II) oxidation, as well as chemolithotrophic S oxidation and aerobic, heterotrophic respiration were integral to catchment terrestrial Fe–C cycling. This work demonstrates bacterial involvement in terrestrial Fe cycling in a subtropical coastal circumneutral-pH ecosystem.

Keywords Forestry management; seasonal water-logging; tidal flushing; bacterial Fe cycling; subtropical Australia

1. Introduction

Large-scale exotic *Pinus* plantations were established in subtropical coastal Southeast Queensland (SEQ, Australia) in the 1950s, with forestry practices such as clear-felling and replanting common in recent years. Associated forestry practices are of concern due to potential increases in soil organic

carbon (C) and iron (Fe) bioavailability, which may enhance Fe–C biogeochemical cycling during flooding events and promote Fe mobilization from soils into solution via abiotic and biological mechanisms [13, 28]. Potential elevation of Fe mobilization from terrestrial into aquatic systems may negatively affect water quality through groundwater-associated biofouling [48]. In addition, excess Fe transport into adjacent estuarine and marine waterways may provide the limiting nutrient for blooms of potentially toxic cyanobacteria such as *Lyngbya* spp. [1]. To assess the impacts of *Pinus* forestry on coastal water quality, the occurrence of Fe forms and biogeochemical factors controlling Fe mobilization in coastal SEQ need to be examined.

In the terrestrial subsurface, Fe naturally occurs as (oxy)(hydr)oxides and undergoes active redox cycling between Fe(II) and Fe(III) at oxic–anoxic interfaces. [32]. Active Fe redox cycling and associated mineral mobilization are typically related to microbial Fe(II) oxidation and Fe(III) reduction, in which phylogenetically diverse *Bacteria* and *Archaea* (collectively referred to as Fe bacteria) capable of Fe metabolism act as an important driver of electron flow [11, 31]. Representative organisms include Fe(II)-oxidizing *Gallionella ferruginea* and *Sideroxydans lithotrophicus*, and Fe(III)-reducing *Geobacter metallireducens* and *Shewanella alga* [11, 31]. However, the role of microorganisms in Fe biogeochemistry of O_2-dependent, circumneutral-pH systems has until recently, been poorly understood. This is largely due to the difficulty in establishing associated laboratory microcosms using unstable, O_2-sensitive Fe(II) species, and/or designing RNA/DNA-based group-specific primers/probes targeting diverse Fe bacterial species in environmental samples. To date, group-specific primers/probes have been tested only for representative genera of neutrophilic Fe(III)-reducers such as *Geobacteraceae*, *Shewanella* and *Geothrix* [10, 44]. Few studies have focused on, designed and used group-specific primers/probes for (micro)anaerobic, neutrophilic Fe(II)-oxidizers [54]. Consequently, the knowledge surrounding *in situ* microbial ecology of neutrophilic Fe bacteria, particularly Fe(II)-oxidizing species, is limited [11]. Culture-based studies involving isolation and characterization of Fe bacteria from natural samples are of significance for improving understanding of associated microbial ecology and developing molecular phylogenetic tools [46, 54].

Due to diverse elemental interactions during Fe, manganese (Mn) and sulfur (S) cycling, Mn and S bacteria can be involved in Fe biogeochemistry via biological or abiotic pathways. For example, most known Fe(III)-reducers are capable of Mn(III) reduction, whereas some sulfate-reducers utilize Fe(III) as an alternative electron acceptor {Lovley, 2006 #211}. Microbially-produced Mn oxides and sulfides

react abiotically with Fe, affecting Fe transport and fate in subsurface soils. Associated biological and abiotic interactions commonly lead to zonation of Fe, Mn and S bacterial populations and accumulation of minerals such as pyrite and ferromanganese compounds in soil/sediment redox transition zones [4, 7, 36]. However, recent Fe biogeochemistry studies in circumneutral-pH habitats have focused largely on specific Fe bacterial groups in the northern hemisphere, and overlooked associated Mn- and S-bacterial involvement [5, 55]. To date, there are no studies on the ecology of Fe and Mn or S bacteria in subtropical coastal circumneutral-pH ecosystems in the southern hemisphere, where Fe biogeochemistry is potentially of major environmental significance.

This study was conducted in a representative SEQ coastal catchment (Poona Creek), which has recently been selected for studying forestry-associated Fe biogeochemistry [30]. We determined the occurrence of Fe forms and associated, cultivable bacterial populations in a range of soil cores taken from the catchment including well-drained, plantation areas and water-logged, native-vegetation zones. The study assessed the impacts of forestry practices such as *Pinus* plantation clear-felling and replanting, and seasonal water-logging on bacterially-mediated terrestrial Fe cycling within the catchment.

2. Materials and methods

2.1. Study site and soil sampling

Poona Creek catchment (ca. 100 km^2) lies 300 km north of Brisbane on the Fraser Coast, SEQ, Australia (Fig. 1A). Local climate is subtropical maritime with most rainfall occurring during warmer months (Oct−Mar). Annual rainfall is approximately 1 148 mm. Mean monthly maximum temperatures range from 22.5°C in July to 30.7°C in January; mean minimum monthly temperatures range from 8.6°C in July to 20.6°C in January. The catchment is flat and gently undulating, with elevation mostly <50 m above sea level (ASL), except for a few steeper slopes to the west. The catchment is ephemeral in upper-, but perennial in lower-reaches. It is subject to seasonal flooding and drains into the Ramsar- and UNESCO-listed Great Sandy Strait. Bedrock of this region is formed of mudstone, shale, siltstone, sandstone and quartzose sandstone of the Dunkinwilla Group. These Late Triassic–Early Jurassic age sedimentary rocks are overlain by the volcanic Grahams Creek Formation in the east (140 Ma) and remnants of the fluvial Elliott Formation of Palaeogene age in the north. These formations have been highly weathered since the Miocene (24 Ma) and nodular ferricretes are a

prominent feature of many soils in this area. Podzolic soils developed from the old coastal plain consist primarily of poorly-consolidated sand, mud and gravel, with a deep, weathered profile. Red and yellow podzols commonly occur on the upper to mid slopes, with gray podzols on the poorly-drained lower slopes and broad depressions subject to prolonged water-logging [9, 29]. Exotic *Pinus* plantation currently occupies just over half the catchment area (Fig 1B). Strips of native vegetation including *Melaleuca* and *Eucalyptus* spp. serves as buffer zones along waterways near poorly-drained soils.
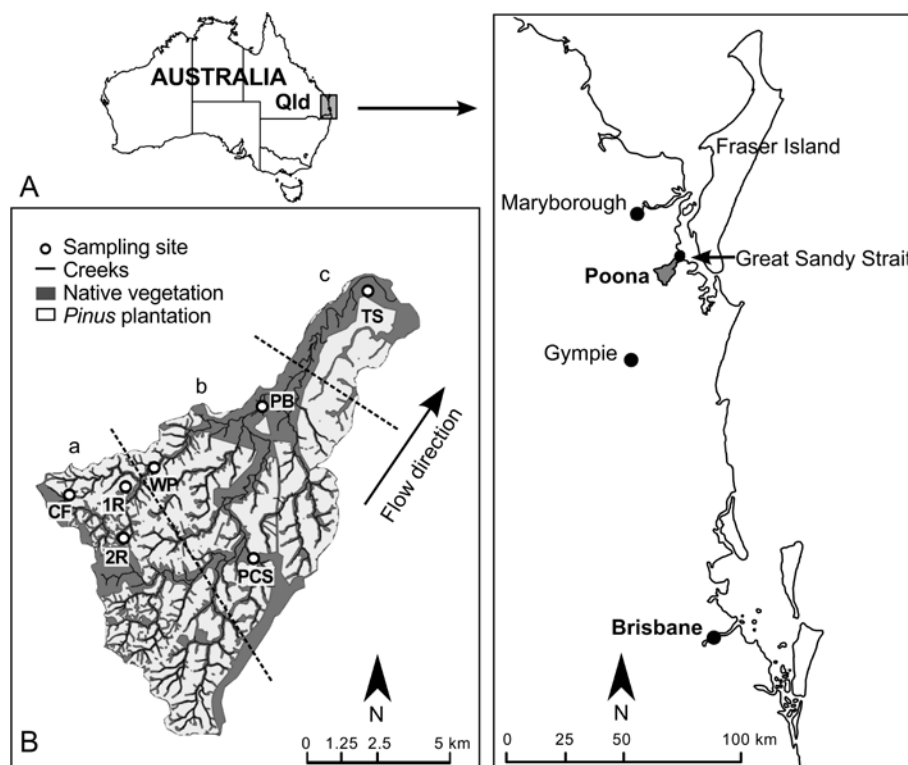

Fig. 1. Location of study area (A) Poona Creek catchment on the Fraser Coast, southeast Queensland; and (B) sampling sites with well-drained plantation soils (1R, mature, first-rotation *Pinus*; CF, *Pinus* clear-felled; 2R, replanted, second-rotation *Pinus*); and water-logged, riparian (WP and PC) and estuarine (TS) soils adjacent to native-vegetation buffer zones.

In December 2008, soils were collected from upper-catchment, well-drained, plantation forest areas associated with mature, first-rotation *Pinus* (1R, ca. 35-year-old), clear-felling (CF) and second-rotation replanting (2R, ca. 5-year-old); and mid-/lower-catchment, water-logged, native-vegetation buffer zones adjacent to stream (WP and PC) and estuary (TS, Fig. 1B). Sites were selected according to catchment soil clusters grouped by Löhr et al. [30] (Table 1). Intact, 30 cm-deep push cores were collected in duplicate at each site using 50 mm internal diameter PVC pipe, sealed with

PVC caps and vinyl electrical tape and transported to the laboratory within 2 d of collection. Cores were subsequently stored at 2–6°C and processed for bacterial cultivation within 14 d. Each core was aseptically divided into three segments by depth (0–10, 10–20 and 20–30 cm), and large plant roots and stones aseptically removed. Soil pH, redox potential (Eh) and electrical conductivity (EC) were measured immediately in the lab using 1:5 (w/v) soil-to-water suspensions and a calibrated TPS 90-FMLV multiple field analyzer [40]. Remaining material was stored at 2–6°C prior to analysis of other physico-chemical parameters within three-months.

Table 1 Poona catchment soil sampling site description.

Sample site	Description [30]
1R	Low-medium Fe areas covering much of upper catchment; both native and plantation
CF	vegetation, commonly situated on steeper slopes; shallow soils with frequent Fe-concretions
2R	which do not release Fe (oxic conditions and moderate pH); outcropping weathered bedrock
WP	High readily-extractable Fe in stream sediment and adjacent riparian soils; presumably microbial reduction of high Fe inputs from various sources, with abundant vegetation litter input; large proportion of total Fe is organically complexed;
PC	High Fe, clay-rich soils in local depressions or low-slope areas subject to seasonal water-logging; cyclic Fe reduction–oxidation, formation of lepidocrocite; Fe-concretions participate in redox cycle; potential areas of Fe accumulation
TS	Deep, quartz sand soil; low Fe and clays, readily-extractable Fe associated with kaolinite

2.2. Physico-chemical and biological analysis

Duplicate 10.0 g subsamples were homogenized and analyzed for moisture content (Wc) by oven-drying (105°C, 24 h) and total organic matter (OM) by loss-on-ignition (2.0 g at 500°C, 4 h) [17, 38]. Particle Volume Distribution (PVD, 0.05–880 µm) was determined using a Malvern Mastersizer S Laser Diffraction Particle Size Analyzer after removal of organic material with H_2O_2 and dispersal of samples with sodium pyrophosphate [22]. Dissolved analytes, including nitrate, phosphate, sulfate and Fe, were extracted as described by Houba et al. [18], and extracts analyzed using colorimetry on a SEAL-AQ2 Discrete Analyzer. The ferrozine method was used to determine dissolved Fe(II) [39].

Labile organic C fractions were analyzed using homogenized field-moist soils. Dissolved organic C (DOC) was estimated by extracting 10.0 g duplicate subsamples with distilled water (1:3 w/v) [45]. Microbial biomass C (MBC) was determined by the chloroform-fumigation-extraction method using 2.64 as the extraction correction factor [52]. DOC and MBC extracts were stored at 2–6°C and –20°C, respectively, and analyzed within 3 d of extraction using a Shimadzu TOC-5000A Analyzer.

Total reactive and bioavailable Fe analysis was performed on field-moist samples using N_2-bubbled dissolution reagents as listed in Table 2. Extracts were processed under a N_2 atmosphere in a glove box and stored at 2–6°C before analysis [39]. Total Fe content of dithionite, oxalate and pyrophosphate extracts was determined using a Varian 220 FS atomic absorption spectrometer within 14 d of extraction. Fe(II) content of oxalate extracts was determined using ferrozine according to Phillips and Lovley [39]. Total reactive Fe was also extracted from oven-dried soils using the aqua regia-extraction method (Table 2), and extracts analyzed using a Varian Vista-MPX Inductively Coupled Plasma–Optical Emission Spectrometer (ICP-OES) within 14 d of extraction.

Table 2 Solid-phase Fe extraction and differentiation methods used in this study.

Fe extract description	Dissolution reagent(s)	Target chemical form of Fe[a]
Dithionite-extractable Fe	Dithionite–ascorbate	Total reactive, including finely-divided, crystalline and poorly-crystalline, amorphous Fe [8]
Aqua regia-extractable Fe	Aqua regia	Total reactive Fe [20]
Oxalate-extractable Fe	Ammonium oxalate–oxalic acid (pH 3)	Potentially bioavailable, poorly-crystalline, including organic and inorganic Fe complex [8]
Pyrophosphate-extractable Fe	Pyrophosphate	Poorly-crystalline, organically-complexed Fe [8]

a. Crystalline Fe was calculated by subtracting oxalate-extractable Fe from dithionite-extractable Fe; poorly-crystalline, inorganic Fe complexes by subtracting pyrophosphate-extractable Fe from oxalate-extractable Fe.

2.3. Bacterial cultivation and enumeration

Culture-based methods were employed for examination of the occurrence of Fe cycling-associated bacterial populations in catchment soils, because selective primers (or probes) targeting specific bacterial physiological groups such as neutrophilic Fe(II)-oxidizers are lacking or considered unsuitable for environmental samples [46, 54]. Laboratory enrichment media used for bacterial cultivation and enumeration are listed in Table 3. Oxic and anoxic sterile soil extraction buffers were used to extract aerobic and anaerobic bacteria from homogenized soils (2.0 g wet weight) at a ratio of 1:10 (w/v), respectively. The extraction included two successive steps of high-speed vortexing of 1 min each. Extraction buffer, which was degassed with N_2 for extraction of anaerobic bacteria, consisted of 0.20% Tween[TM] 20/80, 0.85% sodium chloride (NaCl), 0.01% antifoam, and 0.60% HEPES ($C_8H_{18}N_2O_4S$) (final pH 7.1). For plate counts of heterotrophic (HPC) and Mn(II)-oxidizing bacteria, 10-fold serial dilutions to 10^{-6} were performed using phosphate buffered saline (pH 6.8), and 0.1 mL of each dilution spread-plated onto R2A and PC medium, respectively. For broth media, 1 mL soil extract was inoculated into 9 mL of each respective medium, followed by 10-fold serial dilutions to 10^{-10} in the

same medium. Dilution-to-extinction was used for bacterial cell counting. The highest dilution with positive growth (e.g. 10^{-n}) was scored and converted to bacterial cell numbers (e.g. 10^{+n}) on a dry soil basis. Bacterial numbers were log-transformed for statistical analysis. An uninoculated control was included for each medium. FeS gradient medium was incubated at 25°C in the dark for 14 d. All other media were incubated at 28°C in the dark for 28 d. Medium 9K was agitated at 200–250 rpm on a rotary shaker. Presumptive positive bacterial growth was determined by observing each medium for turbidity and differential growth reaction (Table 3). The presence of bacterial cells was confirmed by light and phase-contrast microscopy (1000✕ total magnification).

Table 3 Laboratory enrichment media used for bacterial cultivation and enumeration.

Medium	pH	Target bacteria	Positive growth reaction	Reference
R2A	7.2	Aerobic, heterotrophic	Bacterial colonies	[41]
PC	7.0	Aerobic, chemolithotrophic Mn(II)-oxidizing	Mn(IV) oxide-coated colonies turn blue with LBB reagent[a]	[50]
9K	2.5	Aerobic, chemolithotrophic, Fe(II)-oxidizing	Red-brown Fe(III) oxide precipitation	[25]
FeS gradient medium	4.8	Microaerophilic, chemolithotrophic Fe(II)-oxidizing	Red-brown colonies attached to tube wall	[16]
Fe(III)-nitrate medium	7.0	Anaerobic, nitrate-dependent Fe(II)-oxidizing	Red-brown precipitation due to Fe(III) hydrolysis	[57]
Fe(III)-EDTA medium	7.0	Anaerobic, heterotrophic Fe(III)-reducing	Ferrozine indicator turns purple due to Fe(II) production	[15]
Sulfur medium	4.8	Aerobic, chemolithotrophic elemental S-oxidizing	pH indicator, Bromophenol blue turns yellow due to sulfate production	[51]
Thiosulfate medium	7.8	Aerobic, chemolithotrophic, thiosulfate-oxidizing	Bromothymol blue turns yellow due to sulfate production	[51]
MP broth	7.0	Aerobic, chemolithotrophic sulfide-oxidizing	Bromothymol blue turns yellow due to sulfate production	[51]
API	7.5	Anaerobic, heterotrophic sulfate-reducing	Black FeS precipitation	[49]

[a] 0.04% leucoberbelin blue in 45 mM acetic acid.

2.4. Statistical analysis

Results are presented as arithmetic means from duplicate subsamples taken from different depths of duplicate cores for each site (n = 4), and expressed on a dry weight basis. An exception was pH, for which the average was taken as –log(average 10^{-pH}). Spatial variations were observed between data collected from duplicate soil cores such as 2R, contributing to large standard errors for several analytes (Fig. 2–4). Non-normally distributed data were log-transformed prior to statistical analysis. ANOVA was performed to determine significance of treatment effects using the General Linear Model (GLM) and Tukey Honestly-Significant-Difference test (HSD) (p <0.05) for multiple comparisons. Vegetation effect was considered not significant according to Löhr et al. [30]. Relationships amongst soil physico-chemical and biological properties were analyzed using Pearson linear correlations, with p <0.05 considered significant. In order to reduce the number of variables and to simplify data visualization, Principal Component Analysis (PCA) was conducted using 36 soil samples (type × treatment × depth) as rows, and physico-chemical and biological analytes (except for sandy fraction and dissolved phosphate) as columns. Components with eigenvalues >1 and those explained >5% of total variance were considered significant. Data analysis was performed using PASW Statistics 18.

3. Results

3.1. Soil physico-chemical properties

The Poona catchment soils are primarily loamy sands (>70% sand and <10% clay). Clay-rich soils (silt loam) were found at specific depths in mid-catchment, riparian native-vegetation zones (PC >10% clay, Table 4). Associated pH was weakly acidic, with well-drained soil pH (5.1–6.2) significantly lower than that of water-logged soils (5.9–7.1) (p <0.05). The Eh of most well-drained soils was >+300 mV throughout 0–30 cm profiles, and remained <+250 mV in water-logged soils (Table 4), with lowest Eh found at 20–30 cm depth of WP (61 mV) and 10–20 cm depth of PC riparian soils (90 mV). Upper-catchment, plantation soil EC was similar to that of mid-catchment, WP and PC riparian soils (4.9–20.2 µS cm^{-1}), but increased 100-to-1000-fold in lower-catchment, TS estuarine soils (4 020–7 675 µS cm^{-1}).

Dissolved nitrate and phosphate levels were 7 µg N g^{-1} and <2 µg P g^{-1}, respectively, while sulfate was present at concentrations of 17–47 µg S g^{-1} in upper- and mid-catchment soils (Table 4). Substantially higher sulfate levels were found in lower-catchment, TS estuarine soils corresponded

with locally high EC. Dissolved Fe levels were <1 µg Fe g^{-1} in well-drained soils and water-logged estuarine soils, with significantly higher levels in water-logged, riparian soils such as WP 0–30 cm depths (3–5 µg Fe g^{-1}) and PC 10–30 cm depths (2 µg Fe g^{-1}) (p <0.05). Fe(II) was detected in dissolved Fe fractions of WP riparian and TS estuarine soils only (<0.5 µg Fe(II) g^{-1}).

Table 4 Poona catchment soil physico-chemical properties

Soil type	Treat-ment	Depth (cm)	Wc (%)	PVD (%)		pH	Eh (mV)	EC (µS cm^{-1})	Dissolved analytes (µg g^{-1})			
				Clay	Sand				NO$_3^-$	PO$_4^{3-}$	SO$_4^{2-}$	Fe
		0–10	4.0	6.8	78.8	6.2	288	17	2	ND^a	26	0.4
	1R	10–20	4.5	8.2	76.3	6.1	301	8.1	2	ND	18	0.2
		20–30	8.8	8.8	72.9	6.0	312	7.7	2	ND	27	0.5
		0–10	6.6	4.7	81.3	5.1	336	19	7	ND	38	0.5
Well-drained soil	CF	10–20	6.9	5.8	80.6	5.5	325	8.6	5	ND	28	0.4
		20–30	7.6	4.8	82.4	5.8	319	7.9	5	ND	22	2
		0–10	13.1	2.9	82.6	5.3	309	7.2	3	2	17	0.9
	2R	10–20	8.6	5.1	82.0	5.8	296	4.4	3	ND	20	0.5
		20–30	9.2	7.6	79.0	6.1	327	4.9	3	ND	47	0.3
		0–10	35.5	2.4	87.6	6.3	203	17.4	3	ND	36	5
	WP	10–20	22.7	3.5	87.9	6.4	152	12.2	4	ND	24	5
		20–30	21.6	7.7	78.1	6.3	61	20.2	2	ND	26	3
		0–10	39.9	20.3	59.9	5.9	139	9.6	2	ND	32	0.2
Water-logged soil	PC	10–20	25.4	15.0	73.2	6.1	90	12.4	2	2	28	2
		20–30	19.0	7.6	86.2	7.1	142	6.2	2	2	23	2
		0–10	18.9	4.6	85.4	6.7	228	7 675	3	ND	2 565	0.7
	TS	10–20	16.9	5.7	84.7	6.6	254	4 425	2	ND	1 224	0.2
		20–30	19.4	6.7	83.2	6.5	255	4 020	2	ND	1 240	0.2

^{a} < the Minimum Detection Limit (MDL), 0.02 µg P g^{-1}

3.2. Soil organic C fractions

Upper-catchment, plantation soil OM was <3%, with 1R soil OM one-to-three-fold higher than that of CF and 2R soils (Fig. 2A). By comparison, OM was significantly higher in mid-/lower-catchment, water-logged soils (p <0.05), with largest values found in 0–10 cm top layers of WP and PC riparian soils (4.7 and 5.5%, respectively). Soil OM significantly decreased at 10–20 and 20–30 cm depths

across the catchment (p <0.05), with an exception in 1R plantation soils where OM was distributed evenly throughout the 0–30 cm profile (Fig. 2A).

Soil DOC occurred at similar levels catchment-wide and significantly decreased with depths for most samples (p <0.05) (Fig. 2B). An exception was mid-catchment, PC riparian soil, which had DOC <6 µg C g^{-1} throughout the 0–30 cm profile. MBC also decreased with depth for most soils (p <0.05), except for lower-catchment, TS estuarine soils with consistently low MBC at 0–30 cm depths (<100 µg C g^{-1}, Fig. 2C). Soil MBC was significantly higher in water-logged, riparian and estuarine zones compared with than that of well-drained, plantation areas. Highest MBC was found at 0–10 cm depths of mid-catchment, WP and PC riparian soils (481 and 399 µg C g^{-1}, respectively).
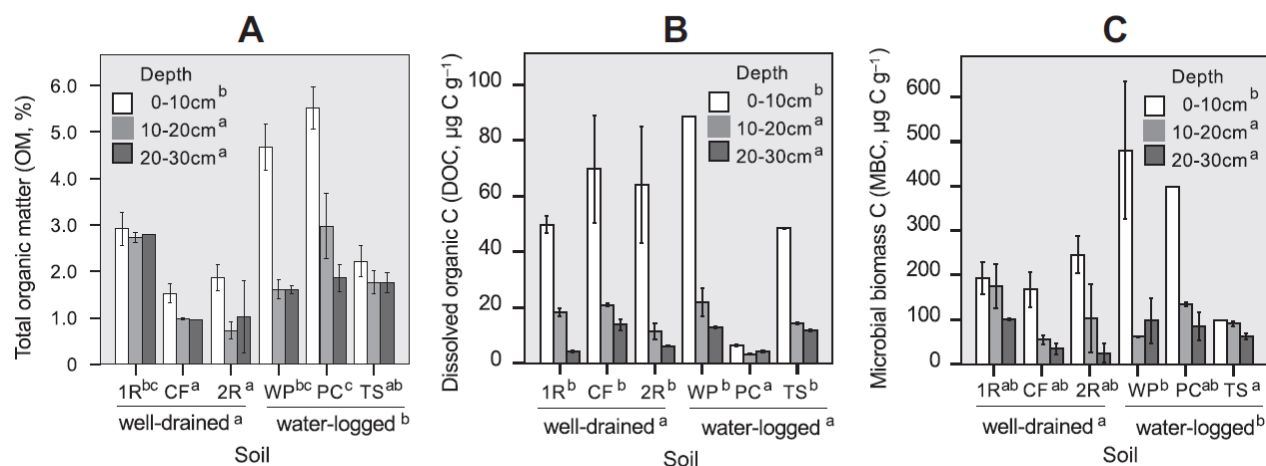

Fig. 2. Poona catchment soil organic C fractions (A) total organic matter; (B) dissolved organic C; and (C) microbial biomass C (bars represent one standard error, n = 4; different superscript letters donate statistically significant variations with depth, site or sample type, p <0.05).

3.3. Total reactive and extractable Fe

Total reactive, dithionite-extractable Fe was abundant and evenly distributed catchment-wide (Fig. 3A), consisting primarily of crystalline forms in upper-catchment, well-drained soils (mean 63.5–90.0%), and less than one half in this form in most water-logged soils in mid- and lower-catchment, riparian and estuarine zones (mean 44.4–45.6%). An exception was PC riparian soil, which had crystalline Fe accounting for 75.0% of total reactive Fe (Table 5). Total aqua regia-extractable Fe comprised one half (or less) of dithionite-extractable Fe in well-drained soils (1.2–6.0 mg Fe g^{-1}), and was similar to dithionite-extractable Fe levels in most water-logged soils (2.9–11.5 mg Fe g^{-1}). However, aqua regia-

extractable Fe exceeded dithionite-extractable Fe levels (9.3–16.5 mg Fe g^{-1}) in PC riparian soils (Fig. 3B).

Poorly-crystalline, oxalate-extractable Fe levels (Fig. 3C) were similar in well-drained 1R soil (1.7–7.0 mg Fe g^{-1}) and water-logged soils (1.3–4.7 mg Fe g^{-1}), two-to-five-fold higher than that in CF and 2R soils (0.5–1.1 mg Fe g^{-1}). The majority of oxalate-extractable Fe (mean 86.4–99.7%) was comprised of organically-complexed, pyrophosphate-extractable forms in well-drained soils (1R, CF and 2R) and water-logged, riparian soils (WP and PC, Table 5). In contrast, TS estuarine soils contained abundant poorly-crystalline, inorganic Fe complexes at 0–10 and 10–20 cm depths, accounting for over half of poorly-crystalline Fe in associated samples (Fig. 3D). Fe(II) was <1.4 µg Fe(II) g^{-1} in poorly-crystalline Fe fractions and did not show a clear trend relating to soil type or treatment (data not shown).
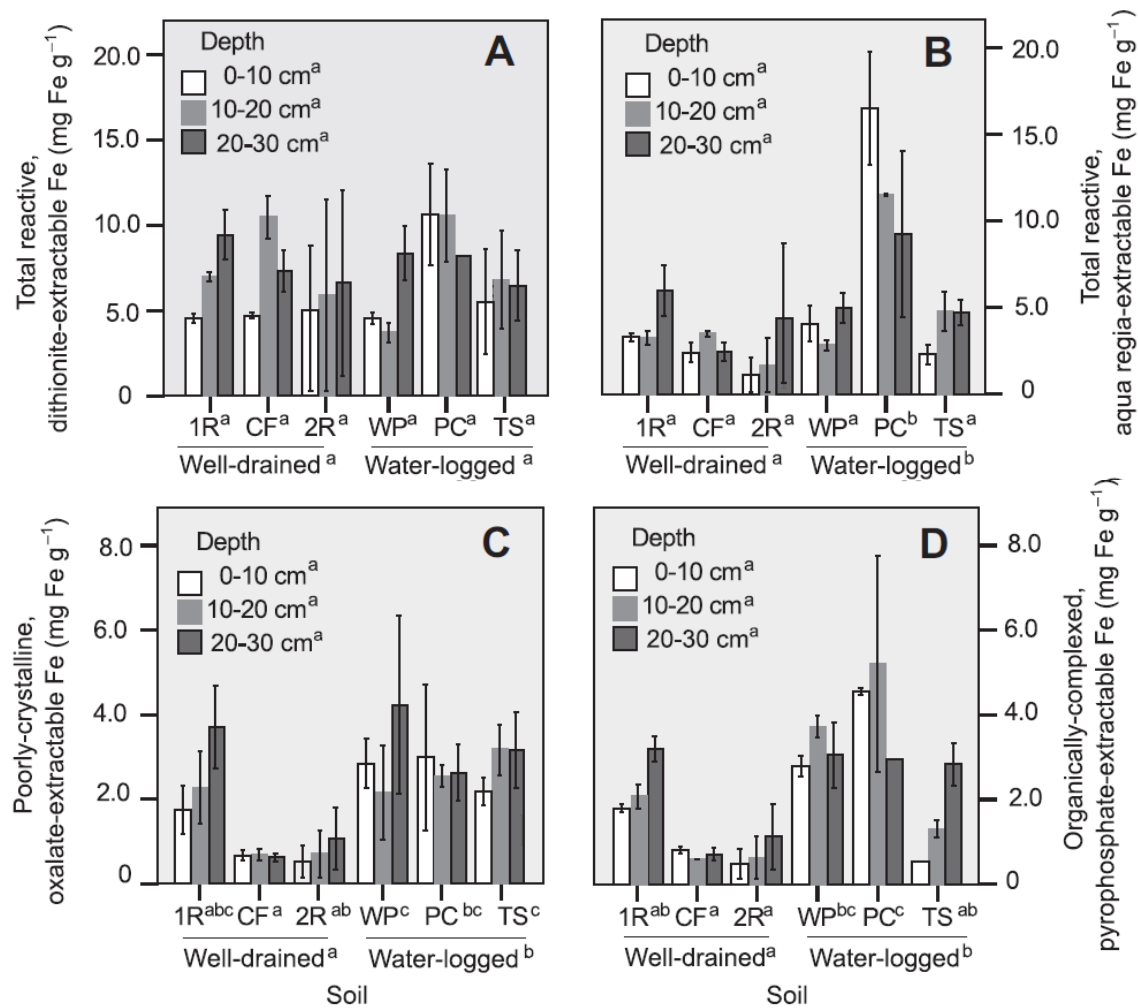

Fig. 3. Poona catchment soil reactive Fe fractions (A) total reactive, dithionite-extractable; (B) total reactive, aqua regia-extractable; (C) poorly-crystalline, oxalate-extractable; and (D) organically-

complexed, pyrophosphate-extractable (bars represent one standard error, n = 4; superscript letters indicate statistically significant difference with depth, site or sample type, $p < 0.05$).

Table 5 Poona catchment soil Fe forms throughout 0–30 cm profiles (mean ± standard error, n = 12)

| Soil type	Well-drained soil			Water-logged soils		
Treatment	1R	CF	2R	WP	PC	TS
Total reactive Fe (mg g^{-1})[a]	7.1 (0.9)	7.5 (1.1)	5.7 (2.3)	5.5 (1.0)	10.1 (1.4)	6.3 (1.2)
% Crystalline form[b]	63.5 (6.5)	90.0 (1.9)	70.9 (8.6)	44.4 (8.8)	75.0 (3.1)	45.6 (11.0)
Poorly crystalline Fe (mg g^{-1})[c]	2.6 (0.5)	0.7 (0.05)	0.8 (0.2)	3.1 (0.7)	2.7 (0.5)	3.4 (0.3)
% Organically-complexed form[d]	86.4 (6.6)	95.2 (4.8)	89.6 (3.8)	87.4 (10.5)	99.7 (0.3)	53.3 (13.1)

a. Dithionite-extractable; b. oxalate-extractable Fe subtracted from dithionite-extractable Fe; c. oxalate-extractable; and d. pyrophosphate-extractable

3.4. Cultivable Fe bacterial abundances

Laboratory cultivation demonstrated the presence of cultivable bacterial populations capable of diverse C, Fe, Mn or S metabolism in catchment soils (Fig. 4). Anaerobic, neutrophilic Fe(III)-reducing bacteria (Fig. 4A), followed by aerobic, heterotrophic bacteria (Fig. 4B), were most abundant. Levels of Fe(III)-reducing bacteria were significantly higher in well-drained soils and water-logged, riparian soils (ca. 10^6–10^9 cells g^{-1}) compared with those of TS estuarine soils (ca. 10^4 cells g^{-1}) ($p < 0.05$). Similarly, lowest aerobic, heterotrophic bacterial abundances (HPC) were found in TS estuarine soils (ca. 10^4 CFU g^{-1}). Anaerobic, Fe(III)-reducing and aerobic, heterotrophic bacterial levels (log-transformed) did not vary significantly with forestry practices or depth ($p > 0.05$).

Despite a lack of cultivable acidophilic and neutrophilic Fe(II)-oxidizing bacteria catchment-wide (<MDL, 10 cells g^{-1}), aerobic, Mn(II)- and S-oxidizing bacteria were present in most soils, with densities several orders-of-magnitude lower than HPC and Fe(III)-reducing bacteria. Cultivable Mn(II)-oxidizing bacterial levels showed a lack of substantial variation with soil type (Fig. 4C), whereas elemental S-oxidizing bacterial levels were significantly higher in water-logged, WP and PC riparian soils compared with well-drained soils (1R, CF and 2R) ($p < 0.05$). In addition, there were significant increases in elemental S-oxidizing bacterial levels from 1R to CF and 2R soils throughout 0–30 cm profiles ($p < 0.05$) (Fig. 4D).

Cultivable thiosulfate-, and sulfide-oxidizing bacteria occurred at low levels for most samples (<10^3 cells g^{-1}, Fig. 4E, F), whereas sulfate-reducing bacteria were found in water-logged, native-vegetation soils at specific depths with Eh <+250 mV (Fig. 4G). Levels of aerobic sulfide-oxidizing and anaerobic sulfate-reducing bacteria were both highest in WP riparian soils. Low levels of cultivable Mn and S bacteria in TS estuarine soils occurred primarily at 0–10 cm depths. Despite small changes, neither Mn nor S-oxidizing bacterial levels (log-transformed) varied significantly with forestry practices or depth (p >0.05) (Fig. 4).
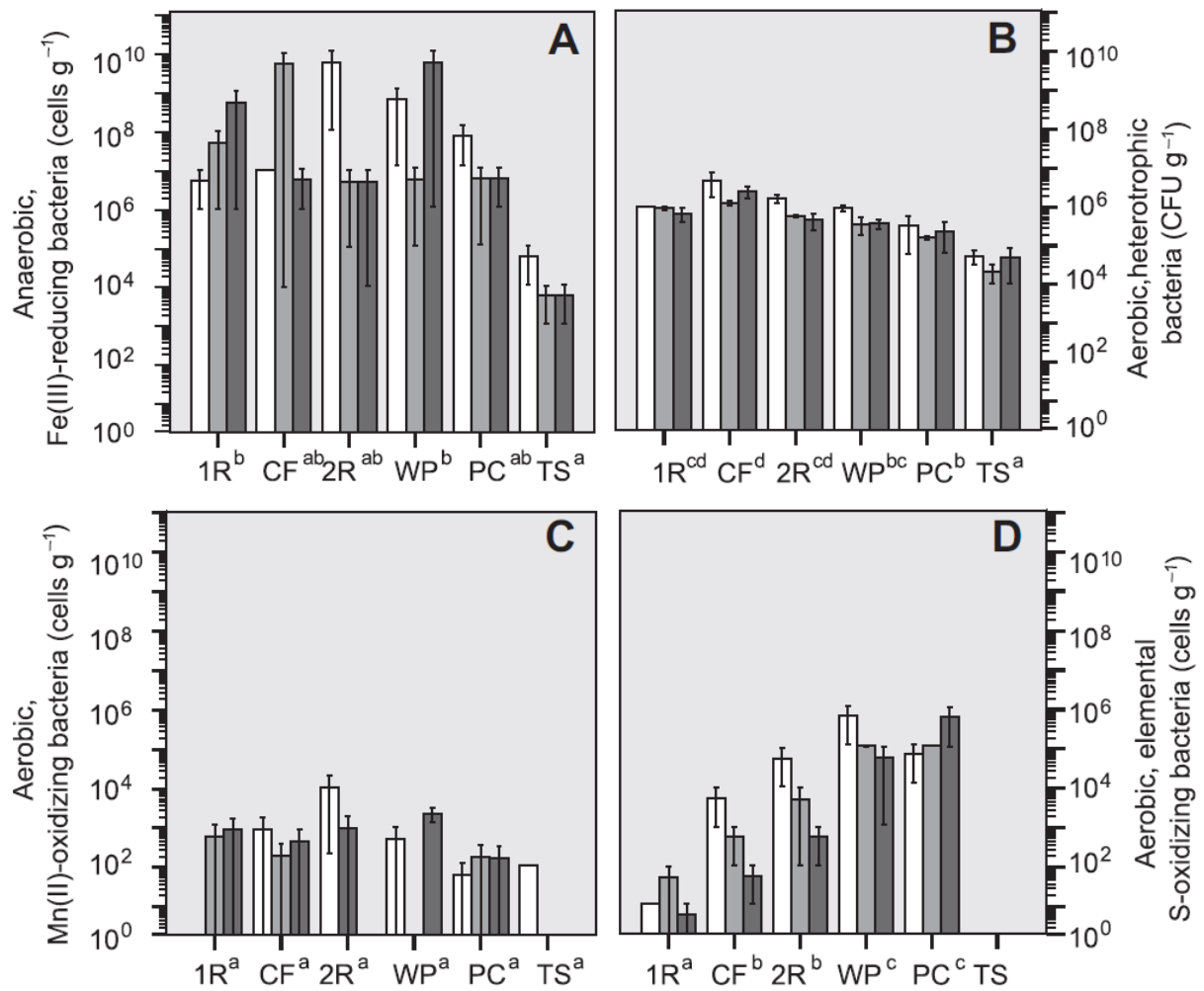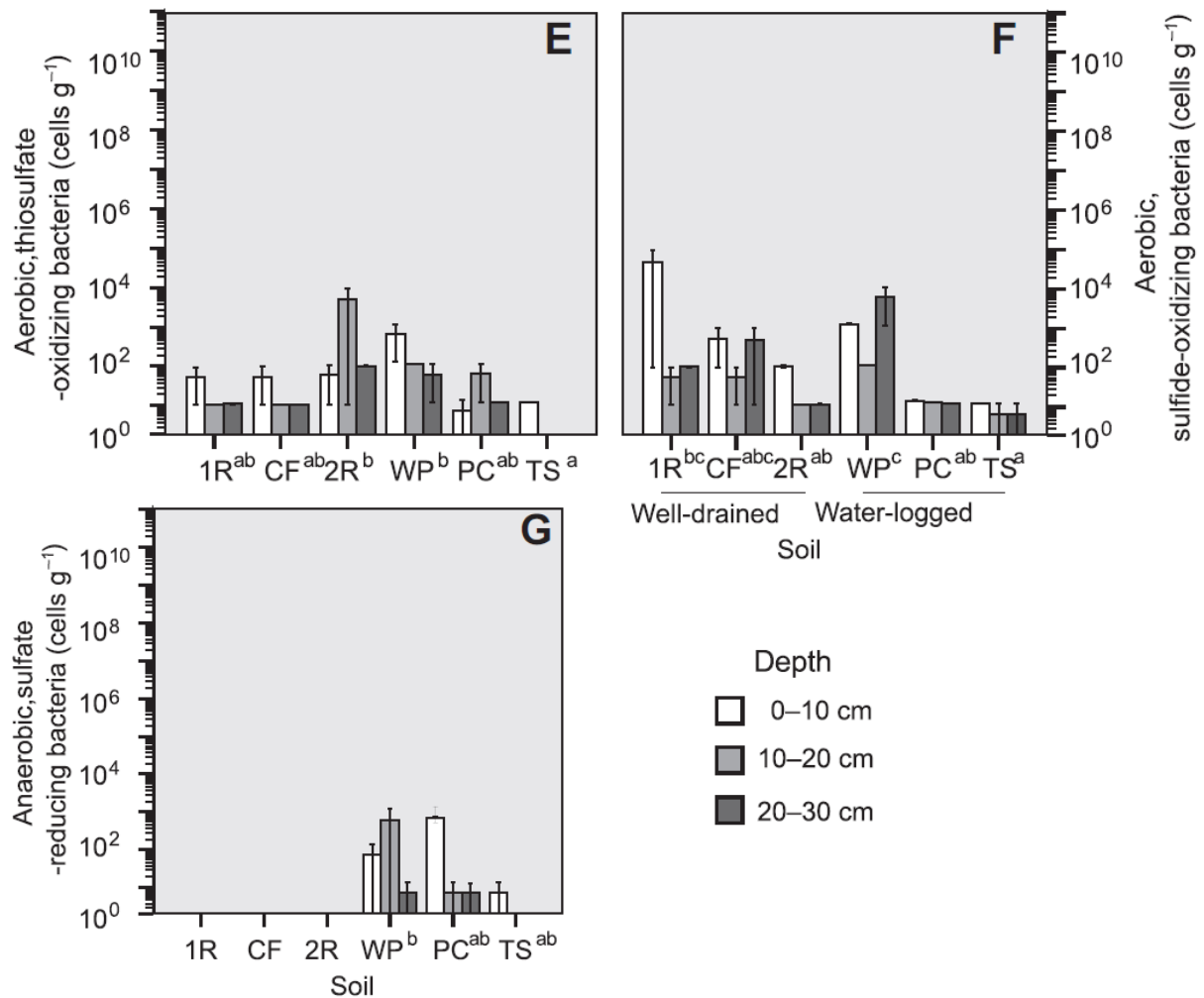
Fig. 4. Poona catchment soil cultivable bacterial numbers (A) anaerobic Fe(III)-reducing; (B) aerobic, heterotrophic; (C) aerobic Mn(II)-oxidizing; (D) aerobic, elemental S-oxidizing; (E) aerobic, thiosulfate-oxidizing; (F) aerobic, sulfide-oxidizing; and (G) anaerobic, sulfate-reducing (bars represent one standard error, n = 4; superscript letters indicate statistically significant difference with sample type or site/depth, $p < 0.05$).

3.5. Relationships amongst soil physico-chemical and biological properties

Across the Poona catchment terrestrial system, significant correlations were observed between soil OM and MBC ($p < 0.01$), DOC and MBC ($p < 0.05$), as well as aqua regia-extractable and dithionite-, oxalate- and pyrophosphate-extractable Fe ($p < 0.01$). Reactive Fe fractions including dissolved Fe were positively correlated with moisture and negatively correlated with Eh ($p < 0.01$). An exception was total dithionite-extractable Fe, which was related to clay fraction and DOC ($p < 0.01$). In addition, significant correlations were observed between levels of HPC and thiosulfate-oxidizing and

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Fe(III)/sulfate-reducing bacteria ($p < 0.01$), Fe(III)-reducing bacteria and elemental S-/sulfide-oxidizing bacteria ($p < 0.05$), as well as elemental S-oxidizing bacteria and thiosulfate-oxidizing and sulfate-reducing bacteria ($p < 0.05$). HPC and sulfide-oxidizing bacterial levels were positively correlated with DOC ($p < 0.05$), whereas elemental S-oxidizing and Fe(III)-/sulfate-reducing bacterial levels were related to MBC ($p < 0.05$). Elemental S-oxidizing bacterial levels were correlated with pyrophosphate-extractable ($p < 0.05$) and dissolved Fe ($p < 0.01$). No statistically significant correlation was found between levels of Mn(II)-oxidizing bacteria and any soil organic C-type or Fe/S species analyzed.

3.6. Significance of soil physico-chemical and biological parameters accounting for changes induced by forestry practices and seasonal/tidal fluctuations

Five components were extracted from 22 physico-chemical and biological parameters via PCA analysis (Fig. 5). PC1 explained 28.0% of total variance and contrasted Eh, DOC and dissolved nitrate with other parameters such as moisture, OM, MBC, clay and extractable Fe fractions. PC2 accounted for 21.3% of total variance and included six significant, positively weighted parameters reflecting soil biology (MBC and cultivable bacterial levels) and three negatively weighted parameters including EC, dissolved sulfate and pH. PC3, PC4 and PC5 loadings explained 11.2, 8.0 and 6.1% of total variance, respectively. Loading plot of 22 variables based on the first three principal components (60.5% of total variance) demonstrated relationships amongst catchment soil physico-chemical and biological properties (Fig. 5A), consistent with correlation analysis (see 3.5). The ordination of 36 samples in three dimensions according to PC1, PC2 and PC3 scores showed distinct separations between well-drained and water-logged soils, 0–10 and 10–20/20–30 cm depths, as well as among well-drained soils (1R and CF/2R) and water-logged, riparian and estuarine soils (WP, PC and TS) (Fig. 5B).
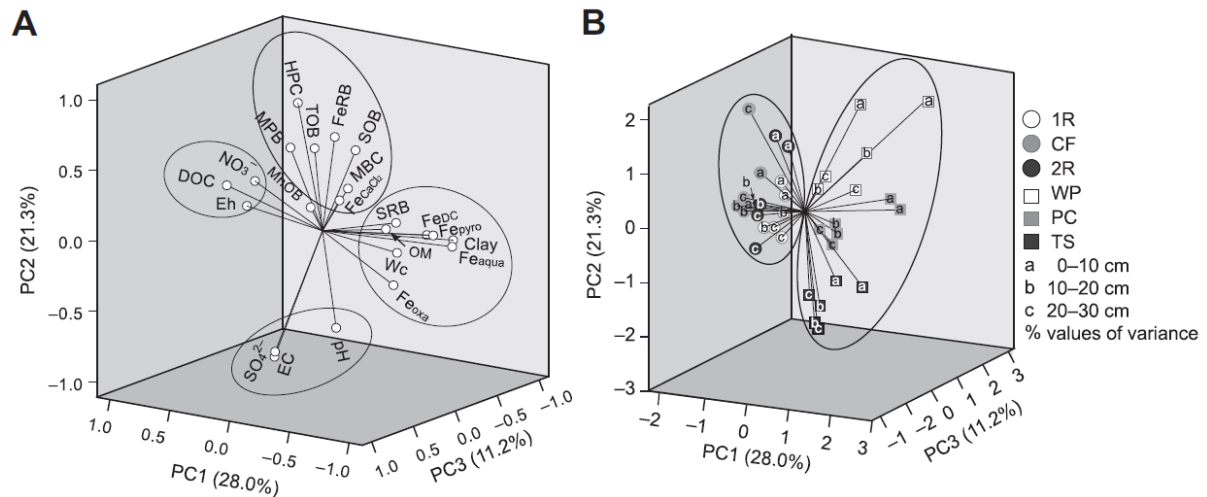
Fig. 5. PCA plot based on the first three components extracted from 22 variables analyzed (A) distribution of catchment soil physico-chemical and biological analytes; and (B) distribution of soil samples. MnOB, Mn(II)-oxidizing; SOB, elemental S-oxidizing; TOB, thiosulfate-oxidizing; MPB, sulfide-oxidizing; and SRB, sulfate-reducing bacterial densities (log-transformed).

4. Discussion

4.1. Impacts of plantation forestry practices on well-drained soils

Poona upper-catchment *Pinus* plantations featured weakly acidic forest soils and oxic conditions (Table 4). Despite reports of soil acidification (pH <4) associated with *Pinus* plantations [2, 26], such an effect was not observed in this study. Levels of soil OM and labile organic C fractions (Fig. 2) were seldom below the values reported by several studies of European soils and an Australian subtropical pine forest soils (2.5–5.5% for OM, 100–600 for DOC and 300–950 µg C g^{-1} for MBC) [6, 21, 58]. This could be related to low clay and high sand content of Poona catchment weathered soils characterized by low dissolved nutrients (Table 4) [37]. Mature, first-rotation *Pinus* soil (1R) was found associated with OM accumulation throughout 0–30 cm profiles (Fig. 2A). Significant OM decreases in clear-felled (CF) and replanted soils (2R) were likely caused by leaf litter and plant detritus removal during plantation harvesting and/or site-preparation burning (Fig. 2A). The lack of statistically significant variations in labile organic C fractions such as DOC and MBC (Fig. 2B, C) suggested OM primarily occurred as insoluble fractions in first-rotation soil, not readily available for heterotrophic bacterial metabolism.

Wet-chemical extraction demonstrated the abundance of total reactive, dithionite-extractable Fe in well-drained soils throughout 0–30 cm profiles (Fig. 3A). Although there was no statistically significant

difference, there was an alluvial trend in total reactive Fe with depth, which could be related to weathered shallow soils situated on regolith. Concentrations were comparable to those found previously in a highly-weathered tropical forest soil and typical Ultisol/Entisol paddy soils associated with seasonal Fe cycling [23, 28, 59]. Aqua regia digestion was less effective than dithionite-citrate at dissolving solid Fe from these soils (Fig. 3B), primarily due to the former functioning though protonation and the latter via reductive dissolution and chelation [35]. The majority of total reactive Fe in first-rotation *Pinus* soils was in potentially bioavailable, poorly-crystalline forms and consisted primarily of organic complexes (Table 5), suggesting localized organic Fe complexation due to OM accumulation. Such complexation was unlikely to occur in clear-felled and replanted soils, which contained low OM and poorly-crystalline Fe (Fig. 2A, 3C). Given poorly-crystalline Fe is a primary substrate for bacterial Fe metabolism in terrestrial subsurface [34], mature, first-rotation plantation soils have the potential for elevation of bacterially-mediated Fe cycling.

Anaerobic, neutrophilic Fe(III)-reducing bacteria were found abundant in well-drained plantation soils (Fig. 4A), with levels comparable to or higher than those reported in a wetland-plant root-zone and sedimentary environments [14, 55]. The presence of both anaerobic, neutrophilic Fe(III)-reducing bacteria and aerobic, heterotrophic bacteria in oxic, well-drained plantation soils (Fig. 4B) suggested that micro-scale bacterial Fe(III) reduction, organic C mineralization and mineral dissolution was occurring in soil aggregates. Our results support recent work in the northern hemisphere, which proposed a significant role of microbial Fe(III) reduction in organic C oxidation in non-flooded tropical or upland forest soils [13, 28]. High densities of Fe(III)-reducing bacteria in Poona catchment well-drained plantation areas demonstrated high potential for bacterial Fe(III)-reductive dissolution from soils, particularly upon heavy flooding.

Levels of cultivable Mn(II)-, elemental S-, thiosulfate-, and sulfide-oxidizing bacteria in well-drained plantation soils were several orders-of-magnitude lower than those found previously in water-logged paddy soils associated with active Mn or S cycling [43, 47]. Their presence in well-drained plantation soils indicated potential interactions between bacterial Fe(III) reduction and Mn/S oxidation via biological and abiotic mechanisms. For example, one study demonstrated *in vitro* depletion of microbially-produced dissolved Fe(II) from sediment pore water due to abiotic oxidation by Mn(IV) [33]. Hence, the presence of Mn(II)-oxidizing bacteria in Poona catchment well-drained soils could indirectly accelerate abiotic Fe(II) oxidation, also supporting bacterial Fe(III) reduction. This is despite an

absence of acidophilic or neutrophilic Fe(II)-oxidizing bacteria related to circumneutral-pH conditions and a lack of available Fe(II) substrates. The lack of cultivable, sulfate-reducing bacteria in oxic plantation soils suggested sulfate reduction and sulfide-linked Fe(III) reduction was unlikely to occur *in situ*, as the former process is considered to be driven by bacterial enzymatic activities [53].

In contrast to substantial changes in soil OM and poorly-crystalline Fe pools observed with forestry practices and/or depth, levels of HPC and cultivable bacteria capable of Fe(III) reduction and Mn/S oxidation did not vary significantly (p >0.05, Fig. 4). We could not rule out the underestimation of associated bacterial abundances via culture-based methods. In addition, temporal variations in bacterial diversity and/or activities, which were not examined in this study, potentially affected soil Fe–C biogeochemistry.

4.2. Impacts of seasonal water-logging and tidal flushing on poorly-drained soils

Poona mid- and lower-catchment, riparian and estuarine soils adjacent to native-vegetation buffer zones were subject to prolonged water-logging and characterized by circumneutral-pH conditions (Table 4). Lowest Eh (<+100 mV) at specific depths of riparian soils (WP and PC, Table 4) suggested O_2 depletion and production of low-redox chemical species via bacterial respiration such as Fe(III)/Mn(IV) reduction [12], whereas abundant sulfate in tidally-influenced estuarine soils (TS) was consistent with elevated EC (Table 4), indicating seawater intrusion. Abundant OM in riparian soils was primarily due to substantial accumulation of plant residues (*Pinus* leaf litter and roots, as well as native vegetation) during flooding. However, DOC was found significantly lower in riparian, clay-rich soil (PC, Fig. 3B), which contained substantial crystalline Fe fractions (Fig. 4). This observation suggested surface adsorption of organic species by reactive clay minerals and/or Fe oxides. Riparian soil MBC was found to be twice that of well-drained plantation soils and tidally-influenced estuarine soils, indicating larger and/or more dynamic microbial communities in associated water-logged freshwater environments. Considering the lack of dense mangrove vegetation, we suggest a ca. 2 mm-thick microbial mat covering the surface soil (pers. obs.) contributed to organic C accumulation and microbial biomass production via photosynthesis at the estuarine sampling site [23].

The majority of poorly-crystalline Fe forms in riparian soils was organically-complexed (Fig. 3A, C) indicating Fe complexation by OM under prolonged water-logged conditions. Indeed, soils collected from Poona streams and adjacent riparian zones have been found consistently related to higher levels

of Fe bound to organic ligands [30]. Despite a lack of statistically significant difference, total reactive Fe in orange-brown-colored, riparian clay-rich soils (PC) averaged twice that of grey-colored, riparian low-clay sand soils (RF), consisting over half of crystalline forms (Table 5). This may be related to recent findings by Löhr et al. [30], which indicated Fe concretions in clay-rich soils situated in local depressions participate in oxidation–reduction cycling, promoting Fe accumulation during seasonal water-logging. These authors identified lepidocrocite in Poona catchment clay-rich soils associated with seasonal reduction–oxidation and conspicuous red mottling, whereas other *In vitro* experiments have shown that neutrophilic Fe(II)-oxidizing *Acidovorax* sp. and Fe(III)-reducing *Shewanella* sp. are involved in the formation of lepidocrocite via enzymatic mechanisms [3, 24]. Hence, abundant reactive crystalline Fe forms in riparian clay-rich soils (PC) are also potentially available to support high levels of Fe(III)-reducing bacteria. Different Fe composition (poorly-crystalline and crystalline) and clay mineral abundances (<10% and >10%, respectively) between riparian soils (WP and PC) likely caused variations in aqua regia-extraction efficiency (Fig. 3B). Further mineralogical analysis is needed to confirm associated Fe oxide types, and thus improve understanding of wet-chemical extraction and Fe diagenesis. In addition, less than one half of poorly-crystalline Fe occurred in organically-complexed forms at lower-catchment estuarine sand soil surface layers (Fig. 3D). Increased inorganic Fe stability could be related to tidal flushing, which elevated ionic strength via seawater intrusion [56].

Cultivable Fe(III)-reducing bacterial levels in water-logged riparian soils were found comparable to those in well-drained soils (Fig. 4A). Associated bacterial Fe(III) reduction and organic C oxidation could be highly active due to the abundance of potentially-bioavailable, crystalline and non-crystalline Fe forms. The absence of anaerobic, nitrate-dependent and neutrophilic, microaerophilic Fe(II)-oxidizing bacteria was consistent with a lack of nitrate and Fe(II) substrates. However, associated organisms resembling stalk-forming *Gallionella* and non-stalk-forming *Sideroxidans* have recently been found in a catchment stream (WP), riparian soil surface water (PC) and coastal shallow groundwater (TS) adjacent to our soil sampling sites [27]. Although bacterially-produced Fe(II) can be rapidly oxidized via abiotic pathways in sedimentary environments [33], we can not rule out potential occurrence of low densities of neutrophilic Fe(II)-oxidizing bacteria and underestimation of associated bacterial abundances via culture-based methods which involves soil extraction and cultivation under laboratory conditions.

Several S-oxidizing bacteria, accompanied by low levels of sulfate-reducing bacteria, were found in water-logged soils (WP, PC and TS), indicating bacterial S redox cycling and sulfide-linked Fe(III) reduction. Levels of aerobic sulfide-oxidizing and anaerobic sulfate-reducing bacteria were substantially lower in deeper depths of riparian clay-rich soils (PC loam, Fig. 4F, G). This suggested active Fe redox cycling via bacterial Fe(III) reduction and abiotic Fe(II) oxidation might competitively inhibit bacterial sulfate reduction [42]. Previous work showed sulfate reduction mediates a minor fraction of anaerobic organic C mineralization in Fe(III)-rich freshwater environments, and dominates organic C metabolism in coastal marine and salt-marsh sediments, even those containing abundant Fe [42]. Surprisingly, we observed low levels of cultivable sulfate-reducing bacteria in tidally-influenced estuarine soil 0–10 cm top layer only (Fig. 4G), despite abundant sulfate and poorly-crystalline, inorganic Fe forms. Due to low MBC, HPC and cultivable Fe/S bacterial densities, we suggest abiotic reactions such as chemical exchange related to tidal flushing dominated Fe redox cycling and associated S transformations at the estuarine sampling site.

4.3. Relationships amongst soil physico-chemical and biological properties

A catchment-wide correlation between DOC and HPC (p <0.05) indicated DOC was a readily available substrate for heterotrophic bacterial metabolism, whereas the lack of direct links between OM and cultivable bacterial densities indicated the majority of OM occurred as insoluble forms, not readily available for bacterial metabolism. However, significant relationships between OM and MBC (p <0.01), as well as DOC and MBC (p <0.05) suggested MBC constitutes an important part of OM and contributes to C sequestration within the system through incorporation of DOC into biomass.

Positive correlation between total dithionite-extractable Fe and clay fraction indicated clay minerals (e.g. vermiculite, illite/smectite and kaolinite) were importance Fe sources [30]. As other extractable and dissolved Fe fractions were correlated positively with moisture and negatively with Eh, we suggest Fe reactivity and bioavailability were related to topographic wetness indices. Despite the lack of significant relationships between levels of Fe(III)-reducing bacteria and extractable Fe fractions consisting primarily of Fe(III), indirect links were implied by correlations between levels of elemental S-oxidizing and Fe(III)-reducing bacteria, as well as S-oxidizing bacteria and Fe forms such as poorly-crystalline, organically-complexed and dissolved Fe (p <0.05). Correlations between MBC and levels of elemental S-oxidizing and Fe(III)-/sulfate-reducing bacteria (p <0.05) together indicate associated

Fe and S bacterial populations are key elements of local microbial communities. Bacterial Fe(III) reduction and abiotic Fe(II) oxidation cycling involves complex interactions with S cycling, particularly in water-logged riparian soils containing abundant bioavailable Fe.

Soil clusters grouped by PCA analysis (Fig. 5B) suggested samples were diverse in water-logged, riparian and estuarine areas (WP, PC and TS), where seasonal and tidal flushing, as well as mineral soil properties (e.g. clay fraction abudance) influenced soil Fe biogeochemistry. Separation of samples between mature, first-rotation plantation soil (1R) and clear-felled/replanted soils (CF/2R) indicated impacts of associated forestry practices on Fe biogeochemistry, particularly at 0–10 cm depths. As narrower redox change zone (<10 or 5 cm) has previously been reported in freshwater wetland and coastal sedimentary environments [19, 42], future examination regarding Fe bacterial ecology within catchment soil 0–10 cm top layers at 1–2 cm intervals may be useful in improving understanding of bacterially-mediated catchment Fe cycling as influenced by forestry management practices and seasonal water-logging.

5. Conclusions

This study presents evidence for bacterial involvement in Fe cycling in diverse soils of a subtropical coastal circumneutral-pH system. Results indicated Fe reactivity and bioavailability were primarily controlled by organic Fe complexation in low-clay sand soils situated in upper-catchment, well-drained plantation areas and mid-catchment, water-logged, riparian native-vegetation buffer zones. Cyclic seasonal water-logging promoted crystalline Fe accumulation in riparian clay-rich loam soils, whereas chemical exchange lead to inorganic Fe complexation in lower-catchment, tidally-influenced sand soils associated with seawater intrusion. Our work highlighted the presence of bacteria involved in Fe biogeochemistry, suggesting that bacterial Fe(III) reduction and abiotic Fe(II) oxidation, as well as chemolithotrophic S oxidation and aerobic, heterotrophic respiration were integral to catchment terrestrial Fe-C cycling. Plantation clear-felling and replanting, as well as seasonal/tidal flushing and clay mineral properties significantly impacted soil chemical and biological characteristics related to Fe cycling, despite a lack of significant variations in cultivable Fe(III)-reducing bacterial densities. Results provide supporting information for a companion microcosm study; a evaluation of potential impacts of forestry practices and seasonal water-logging on Fe mobilization, organic C mineralization and greenhouse gas evolution from the catchment terrestrial system.

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